Efficacy of Mycophenolic Acid Combined With KRP-203, a Novel Immunomodulator, in a Rat Heart Transplantation Model

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Background: To explore a more effective and less toxic immunosuppressive strategy in organ transplantation, we recently developed the novel sphingosine-1-phosphate receptor agonist KRP-203. This study examined the efficacy of KRP-203 combined with mycophenolic acid (MPA), an active metabolite of mycophenolate mofetil, in rat heart allografts.

Methods: Heterotopic heart transplantation was performed in a rat combination of DA (MHC haplotype: $RT1^a$) to Lewis ($RT1^l$). The recipients were divided into 12 groups (n = 5–7): Syngeneic (Lewis to Lewis), Vehicle, KRP-203 (0.3 and 1 mg/kg), MPA (10 and 20 mg/kg), 10 mg/kg MPA with KRP-203 (0.03, 0.3, 1, and 3 mg/kg), and 20 mg/kg MPA with KRP-203 (0.3 and 1 mg/kg). MPA, KRP-203, and vehicle were given orally.

Results: The mean days of survival were 5.8 (vehicle), 7 and 7.9 (0.3 and 1 mg/kg KRP-203, respectively), 12.7 and >54.4 (10 and 20 mg/kg MPA), >39.6 and >30.5 (10 mg/kg MPA with 1 and 3 mg/kg KRP-203), >100 and >87.8 (20 mg/kg MPA with 0.3 and 1 mg/kg KRP-203). Histologic and immunohistochemical analysis revealed that diffuse mononuclear cell infiltration (macrophages and T cells), hemorrhage, myocardial necrosis and fibrosis, and expression of endothelin-1, transforming growth factor-β1, monocyte chemoattractant protein-1, interleukin-8, and E-selectin were markedly diminished in the allografts treated with MPA combined with KRP-203. Pharmacokinetic experiments indicated no interaction between MPA and KRP-203, and both combination regimens were well tolerated.

Conclusions: Combination therapy of MPA with KRP-203 has a therapeutic potential as a novel immunosuppressant strategy in clinical transplantation. J Heart Lung Transplant 2006;25:302–9. Copyright © 2006 by the International Society for Heart and Lung Transplantation.

The introduction of calcineurin inhibitors, such as cyclosporine A and tacrolimus (FK506), significantly improved short-term survival after organ transplantation; however, prevention or control of chronic rejection is still a problem to be solved for transplant recipients.[1] The main features of chronic rejection are transplant vasculopathy characterized by neointimal formation and perivascular inflammatory cell infiltration, and transplant vasculopathy is now recognized as the primary cause of allograft failure after the first year after transplantation.[2–4]

Despite advances in organ preservation and immunosuppressive therapy, the incidence of clinically significant transplant vasculopathy at 3 years is approximately 40%, increasing to 50% at 5 years after transplantation.[4] Also, long-term use of calcineurin inhibitors has adverse effects such as nephrotoxicity,[5,6] hypertension,[7] and hyperlipidemia,[8] which could further accelerate the onset of transplant vasculopathy. Thus, the field of immunosuppressive therapy faces the challenge of developing novel agents that are highly selective for their targets of action and free of toxic side effects.

We have recently synthesized a new immunomodulator, KRP-203 (2-amino-2-{2-[4-(3-benzyloxyphenylthio)-2-chlorophenyl]ethyl}-1,3-propanediol hydrochloride; molecular weight, 480.45 Da).[9] KRP-203 has some similarity of molecular structure to FTY720, which has unique

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Submitted July 11, 2005; revised September 19, 2005; accepted September 22, 2005.

This study was supported by research grants from the Ministry of Education, Science, Sports and Culture (#16590667 to M.T. and #16590625 to E.K.) and Daiwa Securities Health Foundation (M.T.).

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 1053-2498/06/$–see front matter. doi:10.1016/j.healun.2005.09.021

biologic effects on lymphocyte trafficking. KRP-203 reduces peripheral lymphocytes and accelerates their homing into peripheral lymph nodes and markedly prolongs skin and heart allograft survival of a minor histocompatibility complex -disparate rat combination (Lewis-to-F344).[9] KRP-203 also prolongs heart allograft survival of a major histocompatibility complex (MHC)-incompatible rat combination, but KRP-203 monotherapy showed only slightly prolonged allograft survival. Interestingly, KRP-203 combined with sub-therapeutic doses of cyclosporine A synergistically prolonged allograft survival even in MHC-incompatible rat heart transplantation,[10] suggesting that this combination therapy provides an excellent option in organ transplantation as long-term immunosuppressive therapy.

Mycophenolate mofetil (MMF) is a potent immunosuppressant that acts to inhibit lymphocyte proliferation by blocking the production of guanosine nucleotides required for DNA synthesis.[4,11] Large randomized controlled trials have shown that MMF is better than azathioprine when combined with calcineurin inhibitors and prednisolone at reducing acute graft rejection after transplantation.[12] In addition, MMF has been shown to have clinical benefits in patients with calcineurin inhibitor-associated nephrotoxicity after organ transplantation.[13] We therefore tested the combination of mycophenolic acid (MPA), an active metabolite of MMF, with a novel immunomodulator, KRP-203, on rat heart allograft survival and determined that combination therapy of MPA with KRP-203 has a therapeutic potential as a novel immunosuppressant strategy in clinical transplantation.

MATERIALS AND METHODS

Experimental Animals and Immunosuppressive Agents

In-bred adult male DA (MHC haplotype, $RT1^a$) and Lewis ($RT1^l$) rats (initial body weight, 200–250 g) were used as donors and recipients, respectively. These rats were purchased from Charles River Japan, Inc. (Yokohama, Japan). They had free access to standard chow and water and were maintained on a 12-hour light and dark cycle. All animals received humane care in compliance with the *Principles of Laboratory Animal Care*, formulated by the National Society for Medical Research, and the *Guide for the Care and Use of Laboratory Animals*, prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH publication No. 86-23, revised 1985). All experiments in this study were performed in accordance with the Shinshu University Guide for Laboratory Animals.

KRP-203 (synthesized by Kyorin Pharmaceutical Co., Ltd.,Tokyo, Japan) was dissolved in distilled water. MPA (Wako Pure Chemicals, Osaka, Japan) was dissolved in 0.5% sodium carboxymethylcellulose, 0.4% polyoxyethylene sorbitan monooleate, and 0.9% benzyl alcohol.

Heterotopic Rat Heart Transplantation and Experimental Protocols

Heterotopic rat heart transplantation using a cuff technique was performed as described previously.[14] The recipients were divided into 12 groups of 5 to 7 in each group ($n = 74$ total): Syngeneic (Lewis-to-Lewis isograft), Vehicle, KRP-203 treatment (0.3 and 1.0 mg/kg) alone, treatment with MPA (10 and 20 mg/kg) alone, treatment with 10 mg/kg MPA combined with KRP-203 (0.03, 0.3, 1.0, and 3.0 mg/kg), and treatment with 20 mg/kg MPA combined with KRP-203 (0.3 and 1.0 mg/kg) (Table 1).

Heart allograft viability was assessed on a scale of 0 to 4, with grade 0, complete arrest; grade 1, remarkably weak; grade 2, weak; grade 3, slightly weak; grade 4, normal. Rejection was defined as a heartbeat of grade 0. KRP-203 and MPA were orally administered from the day of transplantation. Heart graft survival was followed

Table 1. Effect of Mycophenolic Acid Combined With KRP-203 on Rat Heart Allograft Survival

Group (mg/kg)	*n*	Graft survival (days)	MST (days)	Median (days)
Syngeneic	7	>100, >100, >100, >100, >100, >100, >100	>100	>100
Vehicle	5	5, 6, 6, 6, 6	5.8	6
KRP (0.3)	5	6, 7, 7, 7, 8	7.0	8
KRP (1.0)	7	7, 7, 8, 8, 8, 8, 9	7.9*	8
MPA (10)	6	8, 9, 9, 10, 10, 30	12.7*	9.5
MPA (20)	7	18, 26, 34, 50, 53, >100, >100	>54.4*	50
MPA (10) + KRP (0.03)	6	8, 8, 13, 15, 19, 26	14.8*	14
MPA (10) + KRP (0.3)	6	9, 11, 11, 14, 16, 20	13.5*	12.5
MPA (10) + KRP (1.0)	7	8, 9, 11, 12, 37, >100, >100	>39.6*	12
MPA (10) + KRP (3.0)	6	7, 11, 12, 20, 33, >100	>30.5*	16
MPA (20) + KRP (0.3)	6	>100, >100, >100, >100, >100, >100	>100†‡	>100
MPA (20) + KRP (1.0)	6	27, >100, >100, >100, >100, >100	>87.8*	>100

MST, mean survival time; MPA, mycophenolic acid.
*$p < 0.05$ vs. Vehicle, †$p < 0.01$ vs. Vehicle, ‡$p < 0.05$ vs. MPA (20).