

## FTY720, a new immunosuppressant, as rescue therapy in mouse cardiac transplantation

WANG Ming-Hui<sup>1,2</sup>, Vitaliy MILEKHIN<sup>3</sup>, ZHANG Hua<sup>4</sup>, HUANG Hong-Zheng<sup>4</sup>

<sup>1</sup>Department of Nephrology, <sup>3</sup>Department of Cardiac Surgery, Essen University Hospital, Essen (45122), Germany;  
<sup>4</sup>Department of Cardiothoracic Surgery, the Second Affiliated Hospital, Sun Yat-Sen University, Guangzhou 510120, China

**KEY WORDS** FTY720; rescue therapy; heterologous transplantation; heart; lymphocytes; mice

### ABSTRACT

**AIM:** FTY720 is a new synthetic immunosuppressive agent which has a unique mechanism of action and induces long-term graft acceptance in rat and dog allotransplantation as prophylactic administration. The present study investigated whether FTY720 was able to rescue ongoing acute rejection of solid organ transplants in a mouse heterotopic cardiac transplantation model. **METHODS:** BALB/c hearts were heterotopically grafted in C57BL/6 mice. FTY720, at the doses of 0.5, 1, and 5 mg·kg<sup>-1</sup>·d<sup>-1</sup> or vehicle was administered to recipients once daily by oral gavage from d 3 to d 7 after transplantation. Histological changes of grafts, and the lymphocyte number in the peripheral blood and the peripheral lymph nodes were determined on d 5 after transplantation. **RESULTS:** FTY720 prolonged the median graft survival time dose-dependently and significantly. Histological evaluation revealed less lymphocytic infiltration in cardiac allografts treated with FTY720. Moreover, FTY720 remarkably lowered the number of peripheral blood lymphocytes but significantly increased the lymphocyte number in the mesenteric lymph nodes and the peripheral lymph nodes. **CONCLUSION:** FTY720 used orally as rescue therapy significantly extended allograft survival in mouse heterotopic cardiac transplantation.

### INTRODUCTION

Since its introduction into clinical use, cyclosporin A (CsA) has greatly increased the success of cardiac transplantation<sup>[1-4]</sup>. However, further improvements in immunosuppressive therapy are much needed, because rejection, infection, and drug toxicity remain to be the most common causes of morbidity and mortality in the cardiac transplant population<sup>[5]</sup>. Therefore, considerable effort is being devoted to the development of new,

more effective, and less toxic immunosuppressant agents. Recently, a novel synthetic immuno-suppressant, FTY720 (2-amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-diol hydrochloride), which is a synthetic analog of a natural compound from the fungus *Isaria sinclairi*, has been developed<sup>[6]</sup>. Chemical structure and action mechanism of FTY720 differ from conventional immunosuppressants<sup>[7]</sup>. Although the exact mechanisms of the immunosuppressant have not been fully elucidated, some studies have demonstrated that FTY720 possesses unique immunosuppressive mechanism distinct from CsA<sup>[8,9]</sup>. As prophylactic administration, FTY720 prolonged the survival of skin<sup>[10]</sup>, heart<sup>[11]</sup>, liver<sup>[12]</sup>, kidney<sup>[13]</sup>, small-bowel<sup>[14]</sup>, pancreaticoduodenal<sup>[15]</sup>, and islet allografts<sup>[16]</sup> in rats and dogs without producing any noticeable side effects. However, there are no reports

<sup>2</sup> Correspondence to Dr WANG Ming-Hui. Now in *Department of Cardiothoracic Surgery, the Second Affiliated Hospital, Sun Yat-Sen University, Guangzhou 510120, China.*  
Phn 86-20-8133-2217. Fax 86-20-8133-2853.  
E-mail wangminghui01@hotmail.com

Received 2002-12-16

Accepted 2003-04-23

concerning rescue effects of FTY720 on ongoing process of acute rejection in mouse models. Therefore, in the present study, we initiated FTY720 treatment in a well-established mouse model of cardiac transplantation on d 3 after transplantation, when allografts were ongoing, to evaluate whether FTY720 used as rescue therapy can reverse ongoing acute rejection.

## MATERIALS AND METHODS

**Animals** Male inbred mice from BALB/c (H-2<sup>d</sup>) and C57BL/6 (H-2<sup>b</sup>) strains were obtained from Charles River (Sulzfeld, Germany) and housed at the Animal Center, Essen University Hospital, Germany. Mice weighing between 25 and 30 g were randomly used as donors and recipients. All animals were held under standard conditions at constant temperature, humidity, and light/dark cycles. They were fed with standard diet and had free access to tap water. All animals received care in compliance with the Principles of Laboratory Animal Care and the experimental protocol was approved by the local Animal Care and Research Committee.

**Cardiac transplantation** BALB/C mice served as donors and C57BL/6 mice as recipients. Abdominal heterotopic cardiac transplantation was performed using the modified technique as described<sup>[17]</sup>. In brief, Ketamine (100 mg/kg, CP-Pharma, Burgdorf, Germany) mixed with xylazine (10 mg/kg, Bayer, Leverkusen, Germany) was given by intraperitoneal injection for anesthesia. Donor hearts were perfused with chilled, heparinized saline via the inferior vena cava and harvested after ligation of the vena cava and pulmonary veins. The aorta and pulmonary artery of donor hearts were anastomosed to the abdominal aorta and inferior vena cava of recipients using a microsurgical technique. Ischemic time was routinely 30 to 40 min, with a success rate of approximately 90 %. Technical failures within the first 72 h were excluded from the experiment. The viability of the cardiac allograft was assessed by daily abdominal palpation. The day of rejection was defined as the day of cessation of heart beat. Body weight was recorded on the day of transplantation, weekly, and on the day of rejection and any adverse clinical event was noted.

**Experimental design** FTY720 (Novartis Pharmaceutical Industries, Basle, Switzerland) was dissolved in physiological saline, and administered to recipients once daily by oral gavage (0.1 mL per 10 g body weight).

Transplanted animals were assigned to four ex-

perimental groups ( $n=12$ ) treated either with FTY720 or vehicle. Treatment groups included recipients treated with FTY720 at doses of 0.5 (**F 0.5**), 1 (**F 1.0**), and 5 (**F 5.0**) mg·kg<sup>-1</sup>·d<sup>-1</sup>. Those in the control group (**C**) were treated with vehicle. Treatment started on d 3 after transplantation and continued until rejection or for 7 d.

Six mice from each group were sacrificed at five days after transplantation and the grafts were removed for histological examination. The mesenteric lymph nodes (MLN) and the peripheral lymph nodes (PLN) (axillary LN were used in this study) were harvested and peripheral blood was collected for hemocytometer.

**Histopathological examination** Heart allografts were fixed in 4 % buffered formalin, embedded in paraffin and stained with hematoxylin and eosin. Histopathological examination was performed by 3 observers blinded to all background data. The intensity of mononuclear cell infiltration, myocardial necrosis, and coronary vasculitis were independently graded for severity on a scale of 0 (none present), 1 (minimal grade), 2 (low grade), 3 (moderate grade), and 4 (high grade), and scores were averaged.

**Lymphocyte counts** Peripheral blood lymphocyte (PBL) numbers were counted using a MICROS 60 (AXONLAB AG, Baden-Dattwil, Switzerland). For examination of cell numbers in MLN and PLN, single cell suspensions were prepared by mincing and passing through stainless mesh. The number of total cells in these organs was counted using the MICROS 60.

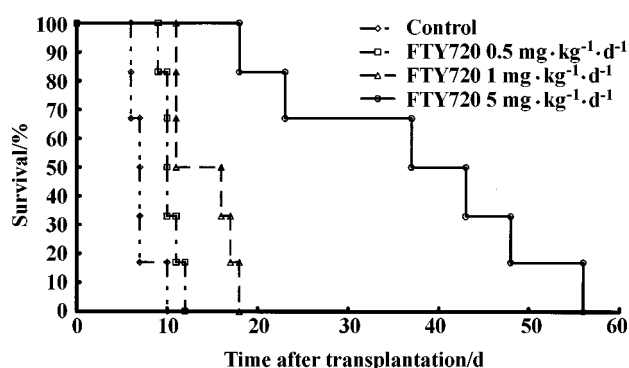
**Statistical analysis** The allograft survival time in different experimental groups was analyzed by Kaplan-Meier technique and compared for statistical significance using the Log-rank test. Scores for histopathological findings were examined by the Mann-Whitney *U* test. In other experiments, data were expressed as mean±SD and statistical differences were calculated by the Student's *t*-test. Differences between groups were considered significant at  $P<0.05$ .

## RESULTS

### Effect of FTY720 on cardiac allograft survival

The saline-treated C57BL/6 recipient mice rejected all BALB/c cardiac allografts within 10 d, with a median graft survival time (MST) of 7 d. When FTY720 was given at the dose of 0.5 mg·kg<sup>-1</sup>·d<sup>-1</sup> from d 3 to d 7 after transplant to treat acute rejection, it effectively prolonged the MST of cardiac allografts to 10 d, as compared with that of the control animals ( $P<0.01$ ). When the doses of FTY720 were raised to 1 mg·kg<sup>-1</sup>·d<sup>-1</sup> with the

same treatment period, they also produced modest extensions of the graft survivals (MST of 11 d,  $P < 0.01$  vs control). Likewise, FTY720 at a dose of  $5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  caused a marked prolongation of graft survival, with an MST of 37 d ( $P < 0.01$  vs control). Furthermore, the MST of the three treatment groups were also significantly different (**F0.5** vs **F1.0**,  $P < 0.05$ ; **F1.0** vs **F5.0**,  $P < 0.01$ ) and there was a dose-dependent effect observed in rescuing cardiac allograft rejections (Fig 1).



**Fig 1.** Effect of FTY720 on the survival of mouse heart allografts in rescuing acute rejection.  $n=6$  in all groups. The graft survival times in FTY720-treated groups were significantly different ( $P < 0.01$ ) to those in the saline-treated group according to the Log-rank test. The graft survival times of the three treatment groups were also significantly different (FTY720  $0.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  vs FTY720  $1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ ,  $P < 0.05$ ; FTY720  $1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  vs FTY720  $5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ ,  $P < 0.01$ ).

The body weight of the animals, recorded on the day of transplantation, weekly, and on the day of rejection was not significantly different between any of the treatment groups. No clinically detectable infections and no mortality occurred during the treatment period in any of the mice.

**Histopathological examination** In comparison with the saline-treated mice, treatment with FTY720  $5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  had a significant effect on mononuclear cell infiltration, vasculitis, and necrosis ( $P < 0.01$ ). Although the grafts of mice treated with FTY720  $0.5$  or  $1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  tended to have a slightly improved rejection score as compared to those in controls, the difference was not significant ( $P > 0.05$ ). Furthermore, Grafts rejected under FTY720 therapy showed a dose-related tendency of lower interstitial and perivascular mononuclear cell infiltration (Fig 2).

**Effect of FTY720 on lymphocyte number in peripheral blood and lymph nodes** FTY720 decreased the number of the peripheral blood lymphocytes (PBL)

significantly and dose-dependently in allografted mice. On d 5 after transplantation, the number of PBL in all FTY720-treated groups was significantly lower than that in the saline-treated group ( $P < 0.01$ ). The  $5.0 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  dose of FTY720 had clearly a maximal effect on the PBL (Fig 3). However, FTY720 did not cause any clear changes in the number of red blood cells and the granulocyte count (data not shown). Concomitant with the marked lymphopenia, the number of lymphocytes in PLN and NLN was significantly increased in a dose-dependent manner after administration of FTY720 as compared with control (Fig 4). These findings indicate that FTY720 modulates the tissue distribution of lymphocytes in blood, PLN, and MLN in mice.

## DISCUSSION

Although FTY720 has been shown to be powerful by itself or as an adjunct immunosuppressant for prolonging allograft survivals when treatment began immediately posttransplant<sup>[10-16]</sup>, its capacity as a rescuing agent for existent acute rejections was less explored. Yuzawa *et al*<sup>[18]</sup> showed that subcutaneous injection of FTY720 could rescue 60 % of acute renal rejection in canine allografts. However, few investigators have demonstrated the rescuing effect on cardiac allografts in mouse models. In our MHC mismatched mouse strain combination, which induced strongly acute rejection within 10 d, FTY720, administered orally at the time of acute rejection, effectively prolonged the survival of murine cardiac allografts in a dose-dependent manner. These data prove a strong immunosuppressive potency of FTY720 as rescue therapy.

The exact mechanism of FTY720 as an immunosuppressant is not fully understood. FTY720 does not inhibit IL-2 and INF- $\gamma$  production and has little effect on IL-2 and INF- $\gamma$  mRNA expressions in allografts<sup>[19,20]</sup>. These findings suggest that FTY720 presumably possesses a unique immunosuppressive mechanism of action distinct from that of CsA, which is known to inhibit IL-2 production from helper T-cells.

It has been demonstrated that the most striking feature of FTY720 is the induction of a dramatic decrease in number of PBL, especially T-cells, at doses that prolong allograft survival<sup>[21,22]</sup>. As a result, the number of infiltrating lymphocytes in graft tissues is markedly reduced, and their detrimental effects on acutely rejecting allografts are thus diminished. This marked decrease in PBL is thought to be the primary immuno-

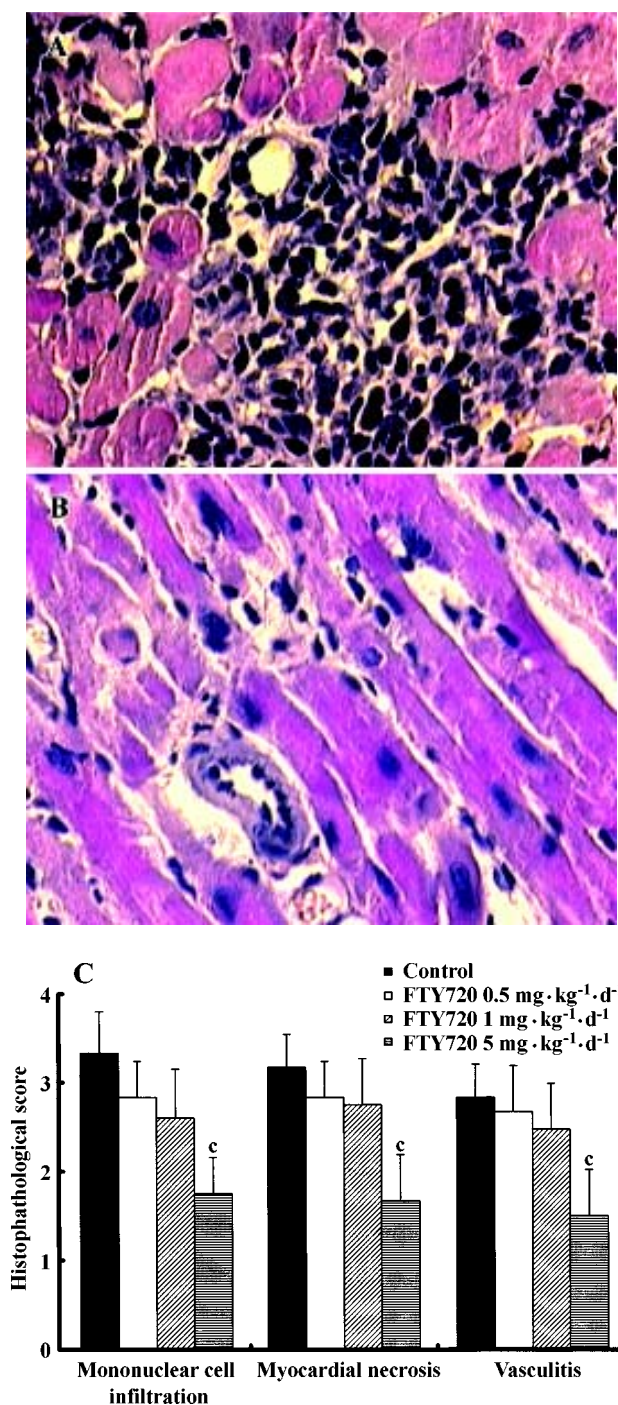


Fig 2. (A, B) Histopathological photos, HE stain, ×150. (A) Control; (B) FTY720 5 mg·kg<sup>-1</sup>·d<sup>-1</sup>; (C) Histopathological scores of mononuclear cell infiltration, myocardial necrosis, and vasculitis 5 d after transplantation. <sup>c</sup>*P*<0.01 vs control.

suppressive mechanism of FTY720<sup>[23]</sup>. The present study showed that oral administration of FTY720 induced significant decreases in PBL dose-dependently. The maximal effect was achieved in the F 5.0 treatment group, in which the number of lymphocytes in

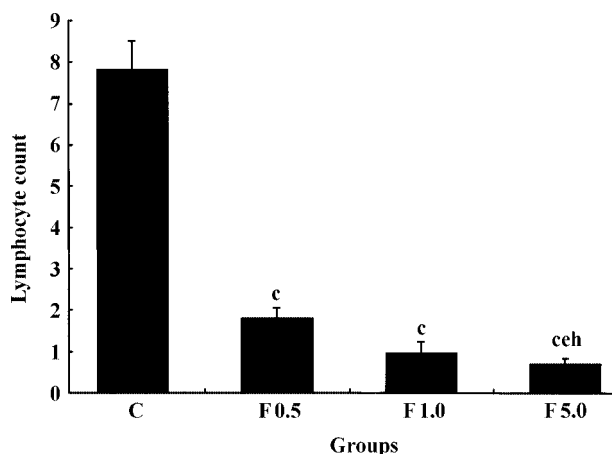


Fig 3. Effect of FTY720 on the peripheral blood lymphocytes. C, control group; F 0.5, FTY720 at 0.5 mg·kg<sup>-1</sup>·d<sup>-1</sup>; F 1.0, FTY720 at 1 mg·kg<sup>-1</sup>·d<sup>-1</sup>; F 5.0, FTY720 at 5 mg·kg<sup>-1</sup>·d<sup>-1</sup>. Lymphocyte count shown in units of 1×10<sup>9</sup> cells/L. <sup>c</sup>*P*<0.01 vs control. <sup>e</sup>*P*<0.05 vs F 0.5. <sup>h</sup>*P*<0.05 vs F 1.0.

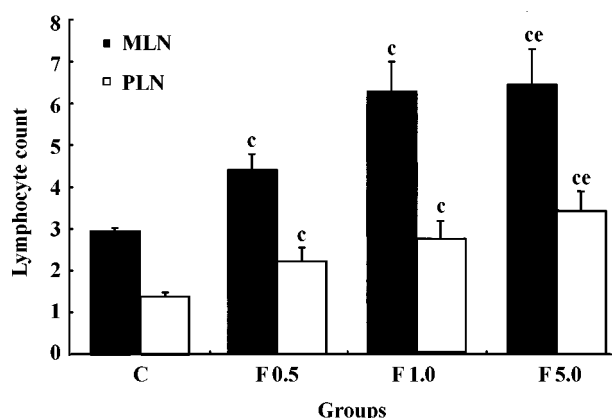


Fig 4. Effect of FTY720 on the lymphocyte number in mesenteric lymph nodes (MLN) and peripheral lymph nodes (PLN). C, control group; F 0.5, FTY720 at 0.5 mg·kg<sup>-1</sup>·d<sup>-1</sup>; F 1.0, FTY720 at 1 mg·kg<sup>-1</sup>·d<sup>-1</sup>; F 5.0, FTY720 at 5 mg·kg<sup>-1</sup>·d<sup>-1</sup>. Lymphocyte count shown in units of 1×10<sup>10</sup> cells/L. <sup>c</sup>*P*<0.01 vs control. <sup>e</sup>*P*<0.05 vs F 0.5.

blood dramatically decreased to less than 10 % of the control values. Furthermore, our histological findings of the grafts revealed that FTY720 reduced lymphocyte infiltration in the rejected grafts. These results are consistent with the above-mentioned reports.

Previous studies have hypothesized that the decrease in lymphocyte number induced by FTY720 is caused by apoptosis of lymphocytes, since FTY720 at high concentrations induced apoptosis of rat splenocytes and human peripheral blood mononuclear cells *in vitro*<sup>[9,24]</sup>.

However, lymphocyte apoptosis may not sufficiently explain the potent immunosuppressive effect of

FTY720 *in vivo*, since the trough level of FTY720 in the peripheral blood of dogs and rats after therapeutic doses is much lower than that sufficient to induce apoptosis *in vitro*<sup>[25]</sup>. Moreover, fluorescently labeled lymphocytes transferred to mice and depleted from the circulation by FTY720 reappeared in the blood after withdrawal of the drug<sup>[26]</sup>. In addition, the number of lymphocytes in PLN, MLN, and Peyer's patches (PP) were conversely increased during the period of marked PBL reduction after oral administration of FTY720<sup>[25]</sup>. Based on these observations, Chiba and his co-workers<sup>[25]</sup> proposed that accelerated lymphocyte homing into lymph nodes (LN) and PP, and subsequent sequestration of circulating mature lymphocytes in these organs were the main mechanisms underlying the immunosuppressive activity of FTY720. In our current allograft model, treatment with FTY720, significantly and dose-dependently, increased lymphocyte counts in PLN and MLN as compared with the control, concomitant with the marked lymphopenia. These findings are in agreement with Chiba's observation.

FTY720 at therapeutic doses does not impair T- and B-cell activation/expansion or memory<sup>[27]</sup>, which may reduce the risk of acquiring infections and virus-related malignancies under immunosuppression. During our experiment, all of FTY720-treated mice without prophylactic administration of antibiotics was lack of any clinically detectable infections. This suggests that FTY720 does not affect lymphocyte function.

In conclusion, our study has shown that oral administration of FTY720 should be effective in the rescue therapy for acute rejection in mouse cardiac transplantation and may be effective in human organ transplantation.

## REFERENCES

- Hosenpud JD, Novick RJ, Breen TJ, Daily OP. The Registry of the International Society for Heart and Lung Transplantation: eleventh official report—1994. *J Heart Lung Transplant* 1994; 13: 561-70.
- Conrad SA, Chhabra A, Vay D. Long-term follow up and complications after cardiac transplantation. *J La State Med Soc* 1993; 145: 217-220, 223-5.
- Oaks TE, Wallwork J. Cardiac transplantation: a review. *Br J Biomed Sci* 1993; 50: 200-11.
- Yeatman M, Smith JA, Dunning JJ, Large SR, Wallwork J. Cardiac transplantation: a review. *Cardiovasc Surg* 1995; 3: 1-14.
- Nair RV, Morris RE. Immunosuppression in cardiac transplantation: a new era in immunopharmacology. *Curr Opin Cardiol* 1995;10: 207-17.
- Adachi K, Kohara T, Mishina T, Fujita T. Design, synthesis, and structure activity relationship of 2-substituted-2-amino-1,3-propanediols: discovery of a novel immunosuppressant, FTY720. *Bioorg Med Chem* 1995; 5: 853-6.
- Hong JC, Kahan BD. Immunosuppressive agents in organ transplantation: past, present, and future. *Semin Nephrol* 2000; 20: 108-25.
- Napoli KL. The FTY720 story. *Ther Drug Monit* 2000; 22: 47-51.
- Suzuki S, Enosawa S, Kakefuda T, Shinomiya T, Amari M, Naoe S, *et al*. A novel immunosuppressant, FTY720, with a unique mechanism of action, induces long-term graft acceptance in rat and dog allotransplantation. *Transplantation* 1996; 61: 200-5.
- Yanagawa Y, Hoshino Y, Chiba K. The significance of timing of FTY720 administration on the immunosuppressive effect to prolong rat skin allograft survival. *Int J Immunopharmacol* 2000; 22: 597-602.
- Ohba M, Li XK, Kita Y, Enosawa S, Funeshima N, Nagai H, *et al*. Long-term graft acceptance in rat heart transplantation by gene transfection combined with fty720 treatment. *World J Surg* 2001; 25: 391-7.
- Furukawa H, Suzuki T, Jin MB, Yamashita K, Taniguchi M, Magata S, *et al*. Prolongation of canine liver allograft survival by a novel immunosuppressant, FTY720. *Transplantation* 2000; 69: 235-41.
- Ueda H, Takahara S, Azuma H, Kusaka M, Suzuki S, Katsuoka Y. Effect of a novel immunosuppressant, FTY720, on allograft survival after renal transplant in rats. *Eur Surg Res* 2000; 32: 279-83.
- Koshiba T, Ji P, Tanaka K, McMaster P, Van Damme B, Waer M, Pirenne J, *et al*. Tolerance induction with FTY720 and donor-specific blood transfusion: discrepancy between heart transplantation and intestinal transplantation. *Transplant Proc* 2000; 32: 1255-7.
- Suzuki K, Kazui T, Kawabe A, Li XK, Funeshima N, Amemiya H, Suzuki S. Immunosuppressive effect of FTY720 on rat pancreas allograft. *Transplant Proc* 1998; 30: 3417.
- Fu F, Hu S, Li S, DeLeo J, Hoover J, Wang S, Lake P, Shi V, *et al*. FTY720, a novel immunosuppressive agent with insulinotropic activity, prolongs graft survival in a mouse islet transplantation model. *Transplant Proc* 2001; 33: 672-3.
- Corry RJ, Winn HJ, Russell PS. Primarily vascularized allografts of hearts in mice. *Transplantation* 1973; 16: 343-50.
- Yuzawa K, Otsuka M, Taniguchi H, Takada Y, Sakurayama N, Jinzenji Y, *et al*. Rescue effect of FTY720 on acute renal rejection in dogs. *Transplant Proc* 1999; 31: 872.
- Hwang MW, Matsumori A, Furukawa Y, Ono K, Okaa M, Iwasaki A, *et al*. FTY720, a new immunosuppressant, promotes long-term graft survival and inhibits the progression of graft coronary artery disease in a murine model of cardiac transplantation. *Circulation* 1999; 100: 1322-9.
- Yanagawa Y, Sugahara K, Kataoka H, Kawaguchi T, Masubuchi Y, Chiba K, *et al*. FTY720, a novel immunosuppressant, induces sequestration circulating mature lymphocytes by acceleration of lymphocyte homing in rats. II.

- FTY720 prolongs skin allograft survival decreasing T cell infiltration into grafts but not cytokine production *in vivo*. *J Immunol* 1998; 160: 5493-6.
- 21 Chiba K, Hoshino Y, Suzuki C, Masubuchi Y, Yanagawa Y, Ohtsuki M, *et al*. FTY720, a novel immunosuppressant possessing unique mechanisms. I. Prolongation of skin allograft survival and synergistic effect in combination with cyclosporine in rat. *Transplant Proc* 1996; 28: 1056.
- 22 Hoshino Y, Suzuki C, Ohtsuki M, Masubuchi Y, Amano Y, Chiba K. FTY720, a novel immunosuppressant possessing unique mechanisms. II. Long-term graft survival induction in rat heterotopic cardiac allograft and synergistic effect in combination with cyclosporine A. *Transplant Proc* 1996; 28: 1060.
- 23 Enosawa S, Suzuki S, Kakefuda T, Li XK, Amemiya H. Induction of selective cell death targeting on mature T lymphocytes in rats by a novel immunosuppressant, FTY720. *Immunopharmacology* 1996; 34: 171-9.
- 24 Suzuki S, Li XK, Enosawa S, Shinomiya T. A new immunosuppressant, FTY720, induces bcl-2-associated apoptotic cell death in human lymphocytes. *Immunology* 1996; 89: 518-23.
- 25 Chiba K, Yanagawa Y, Masubuchi Y, Kataoka H, Kawaguchi T, Ohtsuki M, *et al*. FTY720, a novel immunosuppressant, induces sequestration of circulating mature lymphocytes by acceleration of lymphocyte homing in rats: I. FTY720 selectively decreases the number of circulating mature lymphocytes by acceleration of lymphocyte homing. *J Immunol* 1998; 160: 5037-44.
- 26 Pinschewer DD, Ochsenbein AF, Odermatt B, Brinkmann V, Hengartner H, Zinkernagel RM. FTY720 immunosuppression impairs effector T-cell peripheral homing without affecting induction, expansion, and memory. *J Immunol* 2000; 164: 5761-70.
- 27 Brinkmann V, Pinschewer D, Chiba K, Feng L. FTY720: a novel transplantation drug that modulates lymphocyte traffic rather than activation. *Trends Pharmacol Sci* 2000; 21: 49-52.