行政院國家科學委員會專題研究計畫成果報告

國科會專題計畫成果報告

NSC Project Reports

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計畫名稱:以 FTY720 治療器官移植之慢性排斥－大白鼠動物模型

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一、中文摘要

本研究在於瞭解一新型免疫抑制劑，FTY720**，對於預防器官移植慢性排斥之療效。實驗方法為採用已為學界所公認的** LEWIS **至** F344 **之大鼠心臟異位移植慢性排斥之動物模型。以小劑量** FTY720**（**0.25-0.5 **毫克/公斤體重/天，餵食** 7 **天）治療之移植大鼠，其平均心跳存活時間為** 46-58 **天，此結果明顯地比無任何治療的對照組（**21.7±9.6 **天）為長（**p<0.05**）。而增加** FTY720 **之劑量時，即以較大劑量之** FTY720**（**1-2 **毫克/公斤體重/天，餵食** 7 **天）治療時，大鼠移植心跳之平均都長於** 100 **天（兩組** p<0.01**）。病理切片檢查顯示以較高劑量之** FTY720 **治療之大鼠移植心臟有較輕微慢性排斥之現象。因此本研究顯示** FTY720 **能有效預防大鼠心臟移植慢性排斥之發生。**

關鍵詞：器官移植、慢性排斥、FTY720

一.二 英文摘要 Abstract

This study elucidated the role of FTY720, a novel immunosuppressant, in prevention of chronic rejection by using a well-established animal model (Lewis to Fisher 344 heterotopic cardiac transplant) of chronic rejection. The rats treated by minimal doses (0.25-0.5 mg/kg/day x 7 days) of FTY720 prolonged the mean survival time (MST) to 46-58 days, which were modestly but significantly longer than that of the control group (without any treatment, MST= 21.7+/-9.6 days, both P < 0.05). Increased dosage of FTY720 (1-2 mg/kg/d x 7 days) dramatically and significantly extended the MSTs to more than 100 days (both groups, P< 0.01). Pathologic examinations of the cardiac allografts showed alleviation in the severity of vascular changes of chronic rejections in the groups treated with 1-2 mg/kg/d x 7 days of FTY720. In conclusion, FTY720 might play an important role in long term acceptance of rat cardiac allografts. Its clinical application may help to prevent chronic rejection.

Keywords: organ transplantation, chronic rejection, FTY720

二、緣由與目的（Introduction）

The success of organ transplantation brings new lives to patients with end stage organ failure. Yet, such success has been shadowed by chronic rejection, which is the main cause of allograft failure 5-10 years after transplantation. Thus far, there are still no effective medications to prevent or treat chronic rejection.

This study tried to elucidate the role of FTY720 in prevention of chronic rejection by using a well-established animal model (Lewis to Fisher 344 heterotopic cardiac transplant) of chronic rejection. We compared the differences among the control group (without any treatment), and the groups treated with ascending doses of FTY720.

FTY720 is a novel immunosuppressant isolated and chemically modified from the culture filtrate of Isaria sinclarii. It has been shown to prolong effectively the survival of rat skin, heart, and liver allografts in models of acute rejection. The exact mechanisms of FTY720 are still under intensive investigation and debate. A few possible explanations included induction of lymphocytic apoptosis, homing of lymphocytes to high endothelial venules, or related to intracellular ceramide-sphingomyelin pathways.

二、材料及方法 (Subjects and Methods)

Animals. Adult male inbred Lewis and F344 rats weighing 160-250 g were perchased from Experimental Animal Breeding Center, National Science Council (Taipei, Taiwan) and cared for under the treatment guidelines of the institutional Animal Welfare Committee. Rats were housed in wire-bottomed cages with controlled light/dark cycles and temperature, and received water as well as chow ad libitum. All operations were performed under aseptic conditions and each animal's postoperative condition was monitored daily.

Drugs. FTY720 was generously supplied by Yushitomi Co. Japan. For in vivo use, the drug was dissolved in buffered physiological saline to the desired concentration, stored at 4C, and administered according to the protocol for each experimental group.

Cardiac transplantation. Heterotopic cardiac transplants were placed intra-abdominally into the aorta and vena cava by a modification of the method of Ono and Lindsey. Cold ischemia time was routinely less than 45 minutes. Cardiac activity was assessed daily by abdominal palpation. Rejection, the end-point of graft survival, was defined as the last day of palpable cardiac contraction. Data presented as mean survival time (MST) +/- SD were analyzed by Kaplan-Meier technique and compared for statistical significance using the log-rank test with Bonferroni tests for comparisons among several groups. A value of $P < 0.05$ suggested statistical significance.

Pathological evaluation. The parameters and severity of chronic rejection evaluated included diffuse, concentric proliferation of intima, endothelitis, vascular wall infiltration, in coronary arteries (H-E stain), and severity of fibrosis shown on PAS stain.

三、結果 (Results)

The rats treated with 0.25 mg/kg/day x 7 days of FTY720 prolonged the MST to 58 days, and the group treated with 0.5 mg/kg/day x 7 days of FTY720 had the MST of 46 days; both groups were longer than that of the control group (without any treatment; MST= 21.7+/-6.9 days; Figure 1). The rats treated with 1-2 mg/kg/day x 7 days of FTY720 significantly extended their MSTs to more than 100 days (both P< 0.01 as compared with that of the control group; Figure 1).

Pathologic examination of the cardiac allografts showed alleviation in the severity of vascular changes of chronic rejections in the groups treated with 1-2 mg/kg/d x 7 days of FTY720. We concluded that FTY720 was effective in preventing chronic allograft rejection.

四、討論(Discussion)

Chronic rejection accounts for most of the allograft failure 10 years after transplantation. Effective medications to prevent or treat chronic rejection are still under investigation. Alloantigen dependent and independent factors intercalated to form the final irreversible changes of chronic rejection. Our results in this study supported the benefits of FTY720 therapy in preventing chronic rejection. In conclusion, FTY720 may play an important role in long term acceptance of rat cardiac allografts. Their clinical application may help to prevent chronic rejection.

五、參考文獻(References)

1. Almond PS, Matas AJ, Gillingham K, et al: Predictor of chronic rejection in renal transplant recipients. Transplant proc 25(1): 936 1998
2. Cramer DV, Qian S Haranhn Io, et al: Cardiac transplantation in the rat. Transplantation 47(3): 414-419 1989.
3. Heemann UW, Tullius SG, Schumann V, Tilney NL: Neutrophils and macrphages are prominent in the pathophysiology of chronic rejection of rat kidney allografts. Transplant proc. 25(1): 937-938 1993.
4. M.E. Billingham: Cardiac Transplant Atherosclerosis. Transplantation Proceedings, VolXIX, No4, Supp15(August), 1987: pp19-25.
5. Mennander A, MB, Tikkanen MJ, et al: Chronic rejection in rat aortic allografts. IV. Effect of hypercholesterolemia in allograft arterioscrosis. J heart Lung Transplant 12: 123-32 1993.
6. Russell ME, et al: Immunology of chronic rejection, effector pathways that regulate vascular thickening. Graft. Vol.1, Suppl. II, pp7-10, 1998.
7. Sarris GE, Mitchell RS, Billingham ME, et al: Inhibition of accelerated cardiac allograft arteriosclerosis by fish oil. J Thorac Cardiovasc Surg 1989-97; 841-55.
8. Sharma VK, et al: chronic allograft nephropathy: a TGF-β_1 centered fibrogenic cytokine paradigm for disease progression. Graft. 1, supp1 II, 25-8, 1998.

9. Tullius SG, Heemann UW, Hancock WW, et al: Long-term kidney isografts develop functional and morphologic changes that mimic those of chronic allograft rejection. Annals surgery 220(4): 425-435 1994.
10. Tullius SG, Heemann UW, Hancock WW, et al: Reversibility of chronic renal allograft rejection. Transplantation 58(1): 93-99 1994.
11. Tullius SGm, Heemann UW, Azumah, PradhamP, Tilney NL: Characteristic signs of chronic allograft rejection can be observed in mechanically mainpulated naïve rat kindeys over the long-term. Transplant proc. 27(3): 2131-2132 1995.

六 圖表 (Figure)