行政院國家科學委員會專題研究計畫成果報告

免疫抑制劑 FK-506 及 cyclosporine 對異體移植神經功能恢復之影響

Evaluation of functional recovery after peripheral nerve allotransplantation on FK-506 and cyclosporine treated rats

計畫編號：NSC 88-2314-B-002-329
執行期限：87 年 8 月 1 日至 88 年 12 月 31 日
主持人：簡雄飛　執行機構及單位名稱:臺灣大學醫學院外科

一、中文摘要

周圍神經因外傷或腫瘤造成缺損時，須取一段自體神經(如:Sural nerve)移植，如此受傷神經元之軸突可藉移植神經內呈管狀的 Basal lamina，重新長回肌肉或感覺受器。但移植神經的來源有限且留下麻木的後遺症，故有研究人員嘗試以冷凍過的異體神經來移植。因為冷凍可以殺死神經內的活細胞(如:Schwann cell)而減低排斥性。

離體研究指出，新一代免疫抑制劑 FK-506 (tacrolimus) 對週圍神經細胞之再生有促進作用，而且在大鼠之坐骨神經夾傷模式(crush model)中也初步証實，FK-506 之劑量對於神經軸突之再生與劑量呈正相關，而免疫抑制機轉相同之 cyclosporine 則並無相同周圍神經促進作用。因為實際上之神經移植為切斷後再接，FK-506 對於神經移植後之功能恢復是否有作用並不清楚，所以本研究中，採用小鼠之冷凍神經異體移植模式，探討於 FK-506 免疫抑制下之異體神經功能恢復情形；結果發現每天皮下打 2mg/Kg FK-506 在二個月時可明顯看出 FK-506 可抑制組織排斥同時可促進神經再生，未打 FK-506 的除有神經排斥、basal lamina 被破壞外亦無神經再生現象。

關鍵詞：神經異體移植； FK-506,

Abstract

This study explored the benefits of FK506 in the setting of nerve allograft. A segment of sciatic nerve from mice of C576L/6 was frozen and thawed repeatedly 5 times then was transplanted into a created defect of sciatic nerve in mice of BALB/C. The treated group (N=6) was subcutaneously injected with FK506 (2mg/Kg) daily for 2 months. The control group (N=6) received vehicle (normal saline) only. By Epon embedding and semithin section, we discovered that only frozen allograft treated with anti-rejecting drug will ensure nerve regeneration through the graft. Besides, FK506 has neurotrophic property, with mechanism unrelated to anti-rejection, which will make itself the best choice in nerve allograft transplantation.

Keywords: nerve allograft； FK-506

二、緣由與目的

肢體之異體移植，一直是移植醫學領域中最具挑戰性的題目，一方面肢體包含多種抗原性極高之複合組織(composite tissues)，一方面肢體移植後功能的恢復，還有賴於所有移植組織之精確解剖與生理重建，另外因同時移植之骨骼所含骨髓所引發之移植物-宿主病(graft-vs-host disease)也是潛在的問題。所以儘管肢體異體移植在動物模式上近年來已有許多突破(30,31)，但離臨床應用尚有一段距離。

於組織相容抗原不相容之肢體移植動物模式中，目前僅有少數免疫抑制劑組合能夠在合理的劑量下防止組織排斥，而各種適用的免疫抑制劑何者對於神經生長有最佳的附加效應，也是現今研究的方向之一。假若能讓移植後肢體儘快恢復功能，對肢體移植後之重建必定有極大的幫助(10)，另一方面，對於神經組織異體移植之應用亦有相當之價值。

新一代免疫抑制劑 FK506

(Tacrolimus) 於細胞免疫上具有與 cyclosporine 相似之作用機制 (21,24-26)，它們皆可選擇性地抑制 T-cell 之增殖作用 (6,12)。和 cyclosporine 相比較，FK506 之作用強度為 cyclosporine 的 10~100 倍 (16)，且所產生之副作用較 cyclosporine 小(5)。分別以 FK506 及 cyclosporine 投與各種器官移植病患 (7,15)(肺臟(14)、心臟(28)、肝臟(19)、腎臟(4)、胰臟以及腸道(20))，結果顯示 FK506 不論是在控制排斥反應或是回復排斥反應上之效果皆優於 cyclosporine；此結果尤其在腸道移植之病患更為顯著(11)，亦有人使用於異體神經移植(3)。

除了顯著的免疫抑制效果外，近期的研究報告指出，FK506 對神經系統有保護作用並可縮短神經功能恢復時間(8,17)。FK506 會和體內之 FK506-binding protein (FKBP) 結合形成 FK506/FKBP complex，抑制 calicineurin (calcium/calmodulin dependent phophatase) 之作用，使 nitric oxide synthase (calcineurin 之受質) 因燐酸化之增加而降低分解活性，致 nitric oxide 產生減少，阻止因 glutamate 作用於 N-methyl-D-asparate (NMDA) receptors 所誘起之神經細胞毒害作用，達到神經細胞保護作用(27)。此外，當 calcineurin 作用受抑制時，一種存在於神經突起，和神經軸突生長有關之蛋白質 GAP-43 (亦為 calcineurin 之受質) 含量有提高之現象(18)。依據目前研究推測，神經軸突再生速度加快應該和 GAP-43 蛋白質含量上升有關。

1995 年 Gold 觀察到 FK506 投與壓傷後之坐骨神經可有效縮短神經功能恢復時間(8)。Gold 將 Spraque -Dawley 品系大鼠之坐骨神經以珠寶商鑷子夾傷，而後投與 FK506 (1 mg/kg) 並觀察神經軸突再生之情形。結果發現 FK506 可加快神經軸突再生之速度，在另一篇由 Wang 所作之後續研究中顯示，這種促進再生的作用與劑量之高低成比例(29)。

截至目前為止，有關研究 FK506 對神經系統影響之報告仍採 cell culture model(22,23) 或 nerve crush model(3,29)。但實際上在進行神經移植、肢體移植或再植時，神經卻是完全截斷的，故我們無法確實得知，在 cell culture 或 nerve crush model 所觀察到之 FK506 對神經系統之影響，會同樣發生在截斷後吻合之神經。

初步結果 FK-506 確能促進切斷重接後神經纖維之再生。

我們在 87 年度的研究中，利用組織學的檢查以及實際功能的恢復來進一步探討一般治療劑量下 FK506 對異體移植神經再生速度與品質之影響，並與 cyclosporine 免疫抑制下之神經移植比較。如果 FK506 加快神經軸突再生速度之作用能在此異體神經移植之研究模式中獲得證實，無異是對神經異體移植、肢體異體移植或再植之臨床應用有極大的價值。

三、結果與討論

We used C576L/6 (B6) sciatic nerve as donor graft after being frozen and thawed 5 times. The nerve graft measured 1 cm and be interpositioned within a created defect of BALB/C sciatic nerves which were anastomosed with 10-O Nylon in two to three stitches. The 12 animals of 8 weeks old were divided into two groups of treated and control groups. Immediately following the completion of allotransplantation, 2 mg/Kg daily of FK506 (Fuzisawa, Japan) suspended in normal saline solution was injected subcutaneously for a period of 2 months.

將各組小鼠於術後 60 天，各隨機選取 6 隻予安樂死，取其右後肢之

坐鎮骨神經進行組織固定染色，做神經組織型態測定分析。

大鼠先以腹腔注射 Nembutal (60 mg/kg body weight)方式麻醉，而後腹腔注射 4% chloral hydrate/heparin 溶液(1 ml/100g body weight)以抗凝。打開小鼠胸腔暴露出心臟，於左心室插入導管，以 100 mmHg 壓力灌入生理食鹽水 5 分鐘繼而以 4% glutaraldehyde 固定液灌流 30 分鐘，之後再持續固定 24 小時。而後取出小鼠之坐骨神經，以緩衝溶液沖洗，用 1% osmium tetroxide 作後固定 3 小時，3 小時後經 ethanol 脫水，以 propylene oxide 為過渡溶液，用 Epon812 包埋，切成厚度為 1um 之超薄切片，並以 toluidine blue 染色而後進行神經組織型態測定分析。

Fig A and B showed contralateral-side normal sciatic nerves semithin sections of control group and FK506-treated group respectively. There was no change of axonal size or myelin thickness in treated group. (original magnification: 20X) However, Fig C and D in low power (20x) and Fig E and F in high power (100X) showed marked difference in regards of axonal regeneration and myelin-thickness. There was marked derangement of basal lamina and empty and ballooning space within the graft of control group. The axonal numbers and myelin-thickness were much better within the graft of treated group. The empty space was much less in the treated group.

Fig G (20X) and I (100X) of control group and Fig H (20X) and J (100X) of treated group showed the axonal regeneration 2 mm distal to the graft was quite good in the treated group in comparison with the control group. (arrows in Fig J showed regenerated myelinated axons) There were lots of cells proliferation (possible Schwann cells) within the distal segment of control group. Besides, many apoptotic nuclei were obvious in the control group (arrows in Fig I) but was never found in the treated group.

故往我們作以下結論：每天皮下打 2mg/Kg FK-506 在二個月時可明顯看出 FK-506 可抑制組織排斥同時可促進神經再生，未打 FK-506 的除有神經排斥、basal lamina 被破壞外亦無神經再生現象。

四、計畫成果自評

完成之工作項目及具體成果：

1. 已熟悉鼠神經移植之顯微技巧。
2. 已熟悉神經組織形態學之分析技術。
3. 須再作小鼠感覺與運動神經功能鑑定。
4. 須再探討cyclosporine對移植神經再生之影響。
5. FK506 效果顯著可用於臨床異體神經移植。

五、參考文獻

1. Bain JR, Mackinnon SE, Humter DA. Functional evaluation of complete sciatic, peroneal and posterior tibial nerve lesions in the rat. Plast Recontr Surg 1989;93:129.

2. Brown CJ, Evans PJ, Mackinnon

SE. Inter-and intraobserver reliability of walking-tract analysis used to assess sciatic nerve function in rats. Microsurgery 1991;12:76.

3. Buettemeyer R, Jones NF, Rao UN. Peripheral nerve allotransplant immunosuppressed with FK506: Preliminary results. Transplant.Proc.27, 1995;2:1877-1878.

4. Birk PE, Cook ME, Schmidt WJ, Chavers BM. Preliminary experience with FK506 in pediatric renal transplant recipients: a single-center report. Transplant Proc. 1996;28(2):993-994..

5. Buttemeyer R, Jones NF, Min Z, Rao U. Rejection of the component tissues of limb allografts in rats immunosuppressed with FK-506 and cyclosoprine. Plast Recontr Surg 1996;97:139.

6. Fealy MJ, Umansky WS, Bickel K, Nino JJ, Morris RE, Ress BH. Efficacy of rapamycin and FK-506 in prolonging rat hind limb allograft survival. Ann Surg Transplant Proc 1994;219:88.

7. Fung JJ, Starzl TE. FK506 in solid organ transplantation. Ther Drug Monit 1995;17:592-5.

8. Gold BG, Katoh K, Storm-Dickerson T. The immunosuppressant FK506 increases the rate of axonal regeneration in rat sciatic nerve. J Neurosci 1995;15:7509-16.

9. Goto T, Kino T, Hatanaka H, Okuhara M, Kohsaka M, Aoki H, Imanaka H. FK 506: historical perspectives. Transplant Proc 1991;23:2716-7.

10. Hewitt CW, Puglisi RN, Black KS. Current state of composite tissue and limb allo-transplantation: do present data justify clinical application? Transplant.Proc.1995;1:1414-1415 .

11. High KP. The antimicrobial activities of cyclosporine, FK506, and rapamycin. Transplantation 1994;57:1689-1700.

12. Hotokebuchi T, Sugioka Y, Arai K, Miyahara H, Kaibara N. Rat Limb Allograft Treated with Cyclosprin or FK506: Immunosuppressive Effects and Complications. J.Jpn.Orthop.Assoc. 1992;66:179-80.

13. Hotokebuchi T, Chin I,Saigawa I, Saugioka Y, Arai K. Study of FK506 Optimal Maintenance Dose in Rat Allo Composite Tissues Transplantation. Jpn.J.Transplant. 1994;.5, 558.

14. Hayakawa K, Hata M, Nishiyama T, Ohashi M, Ishikawa M. Maintenance of unresponsiveness by short-term pulse therapy with FK506 in rat transplantation. Transplant Proc. 1996;28(3):1830-1,.

15. Ikebe S, Masumi S, Yano H, Fukunaga T, Shimizu K, Shin S. Immunosuppressive effect of tacrolimus (FK-506). Bone xenografts in rabbits. Acta Orthop Scand. 1996;67(4):389-92.

16. Kino T, Goto T. Discovery of FK-506 and update. Ann NY Acad Sci 1993;685:13-21.

17. Kuroki H, Ishida O, Hatano E, Murakami T, Ikuta y. Experimental ptudy of FK506 on limb allotransplantation in tats: Nerve regeneration and functional convalescence of long term servival animals. Jpn.J.Transplant. 1993;28:367.

18. Kuroki H, Ikuta Y. Nerve regeneration of vascularized rat limb allograft and functional recovery of long-term graft survivals treated by short course of FK506 or cyclosporine. Transplant Proc 1995;27:348-350.

19. Klintmalm GB. Clinical use of FK506 in liver transplantation. [Review] Transplant Proc. 1996;8(2):974-976.

20. Lee KW, Heeckt PF, Halfter WM, Schraut WH, Bauer AJ. Functional impairment of enteric smooth muscle and nerves caused by chronic intestinal allograft rejection regresses after FK506 rescue. Transplantation 1995;59:159-64.

21. Liu J. Fk506 and cyclosporine, molecular probes for studying intracellular signal transduction. Immunol Today 1992;14:290-5.

22. Lyons WE, George EB, Dawson TM, Steiner JP, Snyder SH. Immunosuppressant FK506 promotes neurite outgrowth in cultures of PC12 cells and sensory ganglia. Proc Natl Acad Sci USA 1994;91:3191-5.

23. Lyons WE, Steiner JP, Snyder SH, Dawson TM. Neuronal regeneration enhances the expression of the immunophilin FKBP-12. J Neurosci 1995;15:2985-94.

24. Morris RE. Mechanism of action of new immunosuppressive drugs. Ther Drug Monit 1995;17:564-9.

25. Parsons WH, Sigal NH, Wyvratt MJ. FK-506—a novel immunosuppressant. Ann NY Acad Sci 1993;685:22-36.

26. Thomson AW, Bonham CA. Zeevi A. Mode of action of tacrolimus (FK506): molecular and cellular mechanism. Ther Drug Monit 1995;17:584-91.

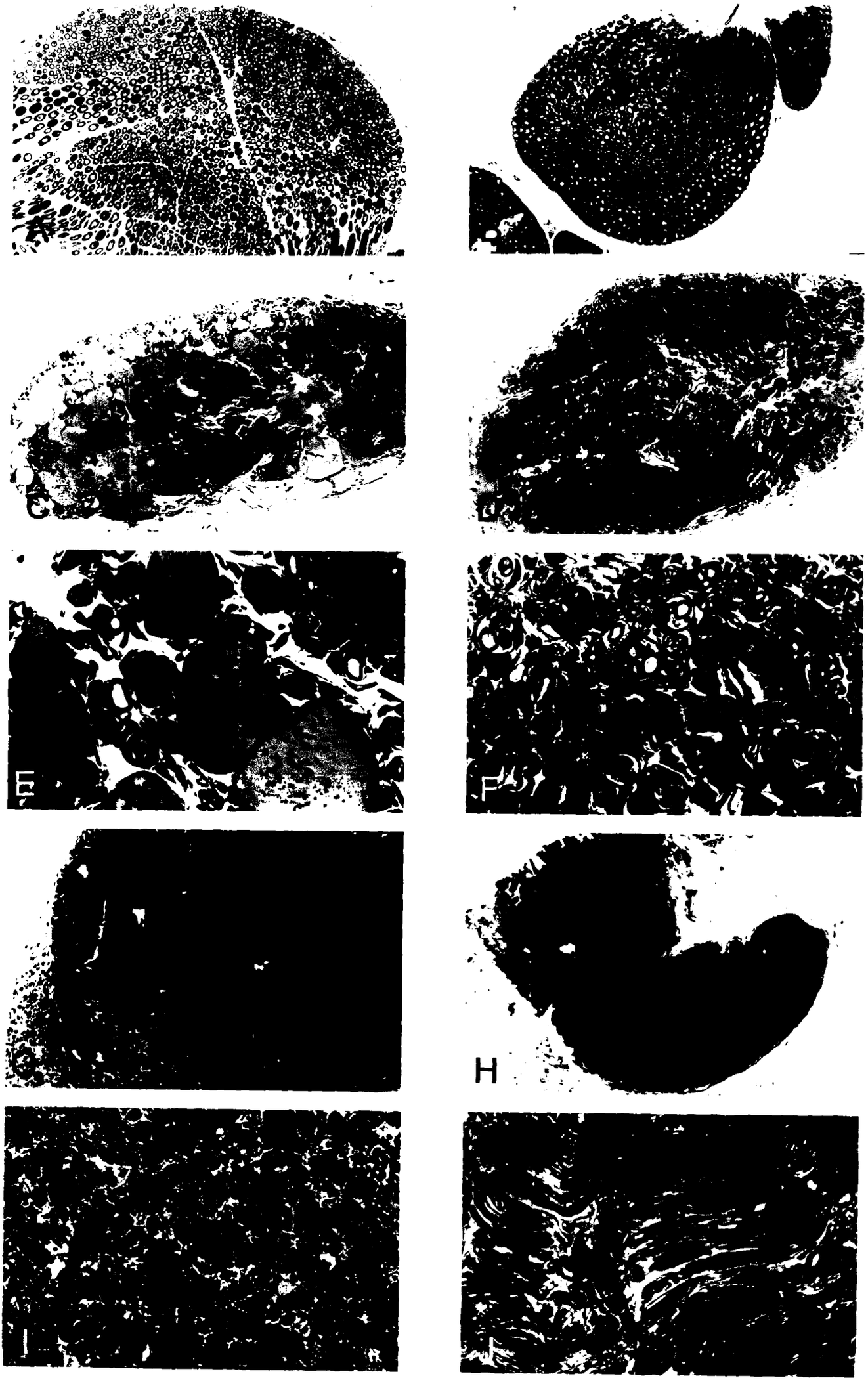
27. Tokime T, Nozaki K, Kikuchi H. Neuroprotective effect of FK506, an immunosuppressant, on transient ischemia in gerbil. Neurosci 1996;206:81-84.

28. Wallemacq PE, Reding R. FK506 (Tacrolimus), a novel immunosuppressant in organ transplantation: clinical, biochemical, and analytical aspects. Transplantation 1993;39:2219-28.

29. Wang MS, Peleny-Pooley M, Gold BG. Comparative dose-dependence study of FK506 and cyclosporin A on the rate of axonal regeneration in the rat sciatic nerve. J Pharm Experi Therap. 1997; 282: 1084.

30. Yeh LS, Gregory CR, Griffey SM, Lecouteur RA, Morris RE. Synergic effect of leflunomide and cyclosporine in rat myocutaneous allotransplantation. Transplantation. 1996; 62:861-877.

[1] 31. Yeh LS, Gregory CR, Griffey SM, Lecouter RA, Hou SM, Morris RE Combination leflunomide and cyclosporine prevents rejection of functional whole limb allografts in the rat. Transplantation. 1997; 64:919-922.


E
F
H