

國科會成果報告

計畫名稱：薑黃素於移植醫學之應用(III)：(1)淋巴球交配反應及細胞素表現(2)預防慢性異種移植排斥及治療急性排斥

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摘要

本計畫主要探討異體移植抗排斥藥物環孢靈(CsA)合併薑黃素(CCM)之後對器官移植預後之改善。實驗結果就以三項表現為主：一、動物移植器官存活，二、細胞素反轉聚合鏈反應 RT-PCR 檢測基因表現，三、淋巴交配反應。

實驗結果：一、動物器官移植

薑黃素對異體心臟移植之體內免疫抑制效果是以大鼠(Brown Norway (heart) to WKY (host))來進行。本計畫中單獨使用薑黃素或是合併次治療劑量環孢靈並監測異體移植心臟存活的平均天數，觀察基準以心跳搏動為準。黃素單獨使用時，顯著地延長平均存活天數(MST \pm -SD)至 20.5 \pm -7.8 (100 mg/d x 14 d 組) 附圖一，及 24.5 \pm -6.3 (100 mg/d x 28d 組) 附圖二，相較於不給藥控制組的 9.1 \pm -1.9 日。合併投與薑黃素及次低劑量環孢靈(10 mg/kg/d x 7d)更使存活天數之延長比兩藥單獨使用(28.5 \pm -5.1 及 35.6 \pm -5.9 天)效果更佳，(P<0.05)。在 BUF x WF 模式，薑黃素單獨使用並未延長平均存活日，但當次劑量環孢靈合併投與，則接近三分之二移植鼠可存活 60 天以上，達長期存活 (P<0.05) 附圖三。

實驗結果：二、細胞素基因調節

適當大小組織以液態氮急速冷凍之後磨成粉狀再加入 Trizol 試劑，轉移 Trizol 混合液到 1.5 離心管並在室溫下放置 10 分鐘。若有不溶物應先去除，加入 chloroform 劇烈混合後以 1.2K 離心 10 分鐘。收集上層水層到新離心管加入 2-propanol 混合並靜置-20 度 C 10 分鐘。以 1.2K 高速離心 10 分鐘後再用 70% alcohol (DEPC)洗過。除去酒精之後風乾並以 100% formamide 溶解，儲存在-70 度 C。反轉錄-聚合鍊反應取用現成試劑以特定引子在 50 度 C 進行 30 分鐘反轉錄反應隨即進行聚合鍊反應。結果使用 2% 瓊脂進行分離，並用標準分子量標記估算。所有進行的細胞素表現結果如圖四。

實驗結果：三、淋巴交配反應

以單向淋巴球交配反應(one-way MLC) 評估薑黃素之免疫抑制效果，從手術取下新鮮脾臟，尼龍網篩出組織細胞液，以密度分層離心法分離淋巴球，以 mitomycin-C 去活化之刺激淋巴球(1×10^5 /孔密度)Brown Norway (BN, RT1ⁿ) 與接受者淋巴球(1×10^5 /孔密度) WKY (RT1^d) 共同培養於一系列遞增濃度下之環孢靈(0 到 1.0×10^3 ng/ml) 與薑黃素(0 到 50 μ M) 之 RPMI1640/10% FCS 96 孔培養盤 37°C; 5 % CO₂ 中 144 小時後，加入 1 μ Ci [³H] thymidine，再 6 小時後收取淋巴球，以閃爍記數儀測量 cpm 作為細胞增值指標。實驗重複三次以上以平均值/標準差表示。

薑黃素可抑制此兩種組織不相容品系鼠 MLR 反應，並呈現濃度依賴性。(ED50=1.44 μ M)，此外薑黃素各濃度下之環孢靈並存於培養液時有相乘效果(synergistic suppressions)。例如，MLC 反應中 [³H] thymidine 結合之閃爍記數值於 10 μ M 薑黃素及 50ng/ml 環孢靈抑制至 (5415 \pm 521 cpm) 相較於任一藥劑單獨使用; 10 μ M 薑黃素 (8297 \pm 2247cpm)；50ng/ml 環孢靈(12982 \pm 1877cpm)(附圖五)。單獨使用及合併使用之 medium effect 亦有明顯增加(附圖六)。在合併用藥指標(CI, combination index) 薑黃素及環孢靈在 MLR 反應中確實有相乘效果(synergistic effect, CI=0.22~0.81, <1)(附圖七)。這對於免疫活化的基本了解中認為 CsA 阻斷與 Calcineurin 有關之 NFAT 活化，而 CCM 阻斷 second signal(CD28)下游之活化機轉的推論是相吻合的。

討論：

三個年度研究之後，我們已建立有系統的大鼠異位心臟移植模式，有助於以後急性與慢性排斥模式下的各種藥物作用分析。

第一部分的異位心臟移植研究結果中，我們首次證明 CCM 併用低濃度之 CsA 能有效延長移植心臟之存活時間，甚至可能造成其長期存活(long-term survival)。此結果指出 CCM 與 CsA 在生體內的確可能有相加或相乘之免疫抑制效果，並值得進一步探討其機轉。

第二部分以淋巴球在藥物調控下受激活後之細胞素(cytokine)分泌研究更可証實此模式之正確性，此部分屬於本計劃第三年實驗，在我們初步採自移植鼠心臟組織樣本，並抽取 RNA 進行 RT-PCR 實驗中，由於技術上的問題目前尚未得到可靠的結論，因此我們計劃先採取組織不相容品系鼠淋巴交配反應的淋巴球細胞作為生體外 RNA 來源(ref 36.)，以期在較易控制的條件下使實驗結果更穩定。

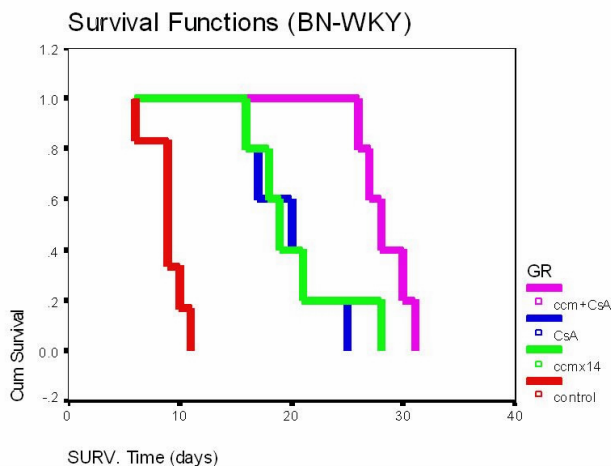
第三部分在淋巴球交配反應中，我們評估了組織不相容品系鼠:WKY(RT1^u) xBN (RT1ⁿ)在一系列遞增濃度的 CsA ” 與/或 ” CCM 的存在下，我們看到以嚴格之 combination index 來評估，CCM 與 CsA 在 MLR 反應中確實有相乘效果(synergistic effect)，這對於免疫活化的基本了解中認為 CsA 阻斷與 Calcineurin 有關之 NFAT 活化，而 CCM 阻斷 second signal(CD28)下游之活化機轉的推論是相吻合的。

展望

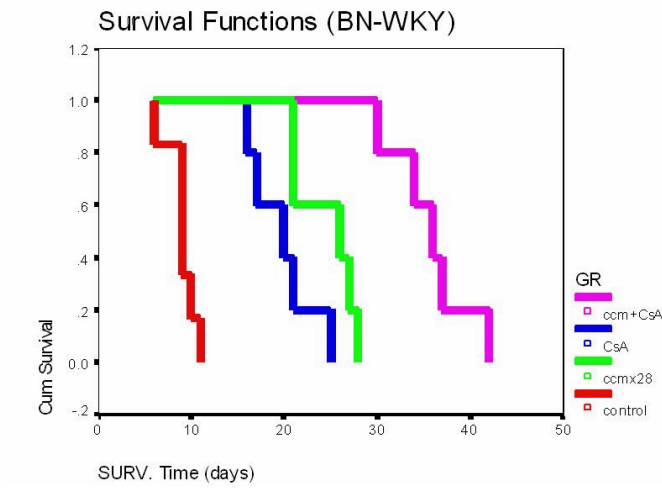
找尋阻斷 CD28 調節途徑並且可以和免疫抑制藥如環孢靈有加成效果的藥是器官移植醫學非常重要的題目。截至目前並沒有用動物模式將薑黃素進行體內試驗的報告，本計畫率先以薑黃素進行此一可能進入醫療行為的理論及其體內表現效果。若是薑黃素可以證實其效果則進一步的臨床人體試驗亦將隨即進行。

附圖

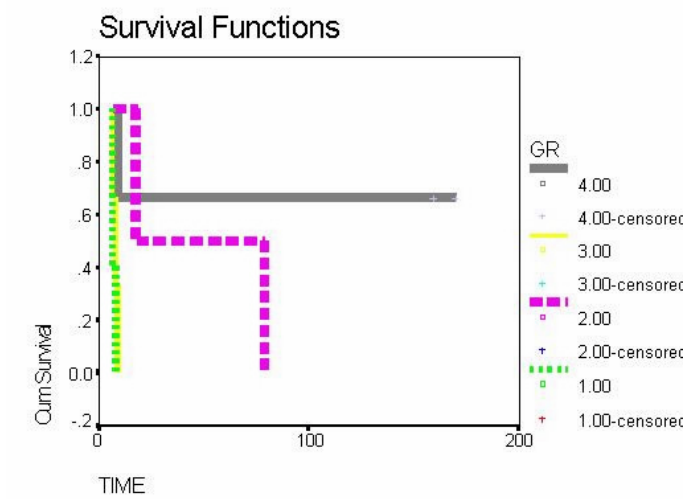
圖一



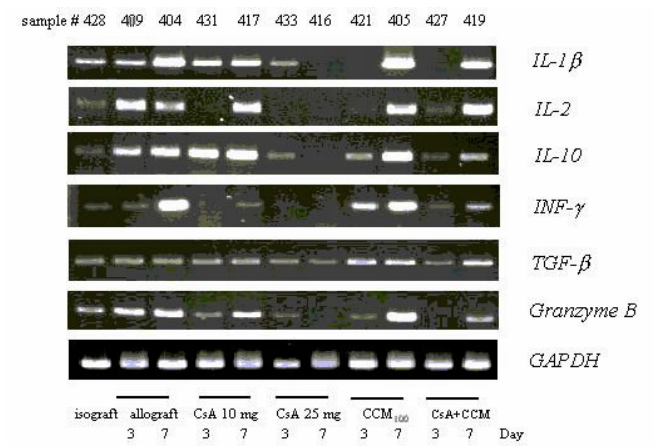
圖二



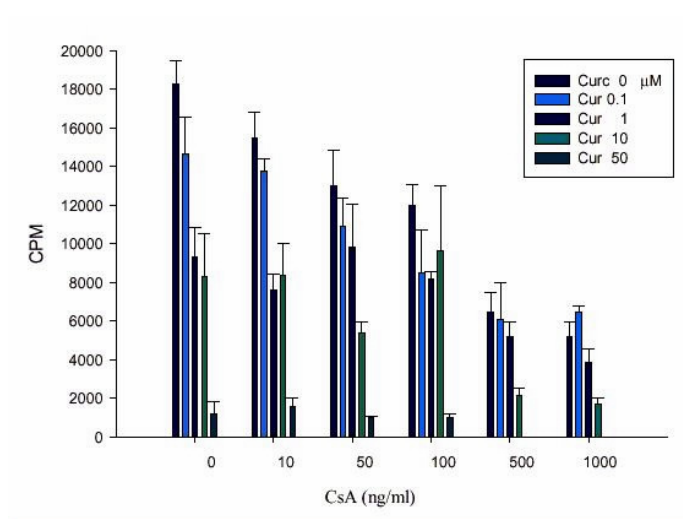
圖三



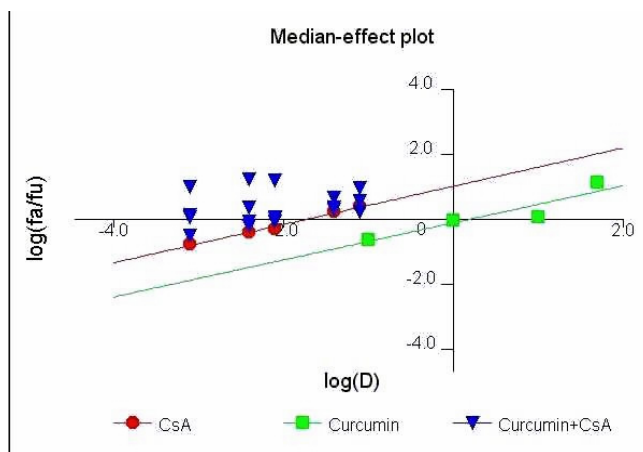
圖四



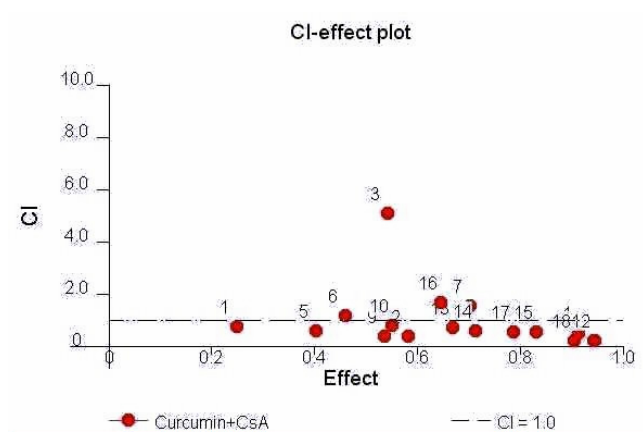
圖五



圖六



圖七



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