行政院國家科學委員會專題研究計畫 成果報告

PAs/MMP-9 酵素系統在小白鼠感染廣東住血線蟲造成嗜伊紅性腦膜炎之致病機轉及治療(2/2)
研究成果報告(完整版)

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目錄

中文摘要

血纖維蛋白酶活化因子(plasminogen activators, PAs)及基質金屬蛋白酶-9 (matrix metalloproteinase-9, MMP-9)在廣東住血線蟲(*Angiostrongylus cantonensis*)所引起的寄生蟲性腦膜炎扮演了重要角色。雖然使用驅蟲藥 albendazole 治療可以殺死在腦部的廣東住血線蟲幼蟲，但是被殺死的幼蟲產生的碎片卻會引起免疫反應造成更嚴重的腦部傷害。過去的研究顯示使用非類固醇類的抗發炎藥物可減輕腦膜炎的病徵。本研究觀察廣東住血線蟲感染後誘發之腦膜炎(meningitis)、細胞激素(cytokines)及蛋白分解酵素(proteolytic enzymes)。腦膜炎之誘發與腫瘤壞死因子(tumor necrosis factor, TNF-α)、介白素-1 beta (interleukin-1 beta, IL-1β)、IL-5、組織型血纖維蛋白酶活化因子(tissue-type PA, tPA)、尿素型血纖維蛋白酶活化因子(urokinase-type PA, uPA)及 MMP-9 呈現正相關。另外，使用 albendazole-thalidomide 混合治療罹患嗜伊紅性腦膜炎的 BLAB/c 小白鼠，並從下列的治療指標中分析治療的效果: (1) eosinophil 的計數。(2) 細胞激素 TNF-α、IL-5、IL-1β。 (3) 蛋白分解酵素 tPA、uPA、MMP-9。研究結果顯示感染廣東住血線蟲第 5 天或第 10 天開始用 albendazole 和 thalidomide 混合治療的組別明顯地減輕了這些治療的指標，且第 5 天及第 10 天開始治療的效果比第 15 天開始治療的效果佳，在同樣的治療劑量及感染時間，較早的治療顯示較佳的結果，顯示治療的時間點與驅蟲藥的藥效有密切的關係。這種使用 albendazole-thalidomide 混合治療的新療法可提供治療寄生蟲性腦膜炎新的方法。

關鍵字: 廣東住血線蟲; albendazole; thalidomide; 組織型血纖維蛋白酶活化因子; 尿素型血纖維蛋白酶活化因子; 基質金屬蛋白酶-9

英文摘要

Plasminogen activators (PAs) and matrix metalloproteinase-9 (MMP-9) proteolytic enzymes may play a role in the pathogenesis of angiostrongyliasis meningitis. Although the anthelmintic agent albendazole can kill the *Angiostrongylus cantonensis* larvae that infect the brain, their dead larvae are capable of evoking a severe, brain damaging immune response. Administration of non-steroid anti-inflammatory drugs have been reported to possibly relieve the symptom of meningitis. The present study was investigated the induction of meningitis, cytokines and proteolytic enzymes after *A. cantonensis* infection. Meningitis was correlated with tumor necrosis factor (TNF-α)、interleukin-1 beta (IL-1β)、IL-5、tissue-type PA (tPA)、urokinase-type PA (uPA) and MMP-9. To observe the curative effects of albendazole-thalidomide co-therapy on eosinophilic meningitis in BALB/c mice. Assay indicators for the therapeutic effect include 1) eosinophil counts; 2) cytokines TNF-α、IL-1β、IL-5; 3) tPA, uPA and MMP-9. The results showed that the albendazole-thalidomide co-therapy significantly decreased (P<0.05) these factors when treated on days 5 or 10 post-inoculation (PI) than treated on day 15 PI. The point of medication is important and is closely related to the anthelmintic efficacy of a drug. At the same dosage and days post-infection, the earlier medication showed better results. This novel therapeutic approach of albendazole-thalidomide co-therapy may provide new methods for treating parasitic meningitis.

Keywords: *Angiostrongylus cantonensis*; albendazole; thalidomide; tPA; uPA; MMP-9

前言

廣東住血線蟲 (*Angiostrongylus cantonensis*) 是一種寄生在大鼠心臟及肺動脈血管的線蟲，屬於人畜共通的寄生蟲 (zoonotic parasites)。主要分佈於東南亞和南太平洋一帶，台灣整個島嶼幾乎都有此

寄生蟲的存在，每年都有因飲食不當而感染廣東住血線蟲的病例報告出現(Yii, 1976; Tsai et al., 2004)。人類的感染主要是由於吃蝸牛肉而意外感染(Alicata, 1965)，此寄生蟲發育中的幼蟲會侵入人類中樞神經系統(central nervous system, CNS)，造成血腦障壁(blood-brain barrier, BBB)破壞，神經細胞脫髓鞘(demyelination) (Hwang et al., 1993)，小腦浦金氏細胞(Purkinje cells)喪失、損傷及空泡化(Perez et al., 1989; Yoshimura, 1993)，嗜伊紅性腦膜炎(eosinophilic meningitis) (Hsu et al., 1990; Ismail and Arsura, 1993)或嗜伊紅性腦膜腦炎(eosinophilic meningoencephalitis) (Gardiner et al., 1990)等病理現象。廣東住血線蟲感染非適當宿主(如人類或小白鼠)，未成熟的成蟲(immature adult)會侵入中樞神經系統(central nerve system, CNS)中，約在感染後三週，腦脊髓液(cerebrospinal fluid, CSF)中的嗜伊紅性白血球(eosinophil)會達到高峰，並伴隨嗜伊紅性腦膜炎的病徵(Sugaya and Yoshimura, 1988; Sasaki et al., 1993)。

血纖維蛋白酶原活化因子(plasminogen activators, PAs)是絲胺酸(serine)家族的蛋白酶，可將血纖維蛋白酶原(plasminogen)活化成血纖維蛋白酶(plasmin)，其活化因子有 tissue-type plasminogen activator (tPA)和 urokinase-type plasminogen (uPA)兩種(Vassalli *et al*., 1991)。目前已知 tPA 會促進 BBB 的破壞且和細菌性腦膜炎的病理變化相關(Busch *et al*., 1997)。此外，uPA 酵素系統也會破壞 BBB 的構造，進而促使白血球浸潤至蜘蛛膜下腔，在病理生理上扮演重要的角色(Winkler *et al*., 2002)。基質金屬蛋白酶(matrix metalloproteinases, MMPs)是一種含鋅的金屬酵素，能分解細胞外基質(extracellular matrix, ECM)，以酵素原型態(proenzyme)產生。MMPs 的不正常表現與許多中樞神經系統疾病有關，例如，多發性硬化症(multiple sclerosis) (Cossins et al., 1997)、阿滋海默症(Alzheimer's disease)、malignant

glioma(Yong et al., 1998)、細菌性腦膜炎(Paul et al., 1998)、病毒性腦膜炎(Kolb et al., 1998)及黴菌性腦膜炎(Matsuura et al., 2000)。本實驗室先前的研究以廣東住血線蟲感染小白鼠，證實 PAs (Hou et al., 2004)及 MMP-9 (Lai et al., 2004; Lee et al., 2004)在廣東住血線蟲感染之嗜伊紅性腦膜炎發生過程中扮演重要角色。另外亦證實嗜伊紅性腦膜炎的發生與 MMP-9 與其天然抑制劑 tissue inhibitor of metalloproteinase-1 (TIMP-1) 失去平衡有關(Chen et al., 2005)。且以免疫電子顯微鏡證實嗜伊紅性白血球(eosinophil)是 MMP-9 的重要來源(Tseng et al., 2004)。

干擾素(interfern gamma, IFN-γ)是重要的炎症介質，具有刺激巨噬細胞活性、調節主要組織相容性復合體 I (major histocompatibility complex I, MHC I)和Ⅱ類抗原表達、調節特異性免疫發育等功能。IFN-γ 的刺激訊號作用可誘導活化細胞中調控增殖相關的訊息蛋白(Janus kinase, JAK)/訊息傳遞與轉錄活化因子(signal transducer and activator of transcription, STAT)通路，使腫瘤壞死因子(tumor necrosis factor alpha, TNF-α)、介白素(interleukin-1 beta, IL-1β)分泌增多，進而誘導核轉錄因子(nuclear factor kappa B, NF-κB)的活化訊號進一步被這些細胞激素增強(Kovarik et al., 1999)。IFN-γ還能藉由加速核因子κB 抑制蛋白(inhibitor of κB, IκB)中 IκB-α降解，促使 NF-κB 與 DNA 的結合，進而促進細胞因子如 TNF-α、IL-1 mRNA 的表現。NF-κB 活化及轉錄受到過氧化自由基、細胞激素、生長因子及急性反應蛋白(acute response protein)刺激細胞內不同之訊息傳遞路徑活化而表現，進而參與細胞的增殖與發炎作用(Baldwin, 1996; Schulze-Osthoff et al., 1997)。在 chondrocyte 細胞，Oncostatin M 會藉由活化 JAK/STAT 路徑誘發 MMP-1、MMP-3、MMP-13 及 TIMP-3 表現(Li et al., 2001)。另外，伯氏疏螺旋菌(*Borrelia burgdorferi*)感染的 chondrocyte 細胞 MMP-1 及 MMP-3 的活化藉由 JAK/STAT 的路徑，且細胞激素 TNF-α 與 MMP-1 活化有關(Behera et al.,

2004)。本實驗室先前的研究以生化分析探討細胞中訊息蛋白質 JAK，STAT，MAP 激酶的激酶(mitogen-activated protein kinase kinase kinase, MEKK)，c-Jun 氨基末端激酶(c-Jun N-terminal kinase, JNK)等分子、核轉錄因子 NF-κB、反應性氧化物質(reactive oxygen species, ROS)與嗜伊紅性腦膜炎發生過程中發炎細胞增殖的作用機制有關(Lee et al., 2000; Lan et al., 2004a)。

Albendazole具有廣泛、高效、低毒的特點，是目前使用最多的廣泛抗蠕蟲藥之一。它選擇性地使線蟲的體表和腸細胞中的微管消失，抑制蟲體對葡萄糖的攝取，減少adenosine triphosphate (ATP)生成，阻礙蟲體生長發育，對多種線蟲的成蟲和幼蟲有殺蟲效果(Venkatesan, 1998)。由於albendazole口服後吸收迅速，肝及肺組織中均能達到相當高的濃度，因此，對腸道外寄生蟲病，如包生條蟲病(hydatid disease)、旋毛蟲病(trichinosis)、中華肝吸蟲病 (clonorchiasis) 及肺吸蟲病 (paragonimiasis)也有好的療效 (Pene et al., 1982; Maisonneucon et al., 1985)。本實驗室先前的研究結果顯示，感染廣東住血線蟲之小白鼠單獨以以驅蟲葯albendazole治療能有效殺死幼蟲，但不能完全抑制因蟲體死亡分解所引起之發炎反應(Lan et al., 2004b)。因此，本研究選擇具抗發炎作用的thalidomide與albendazole混合治療罹患嗜伊紅性腦膜炎之小白鼠。Thalidomide或稱α-phthalimidoglutarimide，它是glutamic acid的衍生物，是白色結晶，無色無味的物質，分子式$C_{13}H_{10}N_2O_4$，分子量 258.23，熔點 271℃，脂溶性，不溶於乙醚及苯，微溶於水，能穿過胎盤 (Winter and Frankus, 1992)。Thalidomide 抑制TNF-α的產生，抑制輔助T細胞(Th1)的活性及增加 Th2 細胞激素 IL-4 和 IL-5 的釋放(McHugh et al., 1995)，它可抑制白血球及單核細胞球的趨化現象(chemotaxis)，具有抗發炎(Miller et al., 1960)、免疫調節及抑制血管增生(anti-angiogenesis)(D'Amato et al., 1994)的作用。最近的研究顯示在痲瘋

結節狀紅斑(erythema nodosum leprosum, ENL)、後天免疫不全症候群(acquire immune deficiency syndrome, AIDS)、癌症及移殖物抗宿主病(graft-versus-host disease, GVHD)、結核病及其他多種疾病，它們的發炎性細胞激素(inflammatory cytokine)TNF-α有顯著的上升，這可能是這些疾病重要的致病原因，因此如能減少TNF-α便可改善由此細胞激素引起的症狀，例如發炎及發燒。在 1950 年代，thalidomide即為廣泛使用的鎮靜安眠藥，在當時因高度的安全性而被應用於預防妊娠性嘔吐。但在上市數年後，發現缺臂、缺眼猶如海豹之畸型兒大增，經由個案追蹤，發現係因懷孕初期婦女服用thalidomide後產下，因此被歐洲、日本、及本國等所禁用。但在 1960 年代，thalidomide意外地被發現具有特殊的免疫調節抑制作用，而重新再被研究並應用於臨床上。目前此藥被用於許多疾病的治療，包括人類免疫缺乏病毒(human immunodeficiency virus, HIV)感染所造成的一些併發症，如口腔潰爛、腹瀉等。

本研究以廣東住血線蟲感染 BALB/c 小白鼠，誘發嗜伊紅性腦膜炎的模式進行研究。第一年將探討 BALB/c 小白鼠腦脊髓液中的 PAs 及 MMP-9 產生的訊息傳遞是否可藉由 cytokines(TNF-α、IL-1β)誘發的 JAK/STAT 路徑。第二年將探討 albendazole-thalidomide 混合治療後，細胞激素(IFN-γ、IL-5、TNF-α、IL-1β)、訊息蛋白質(JAK、STAT、NF-κB)，PAs 及 MMP-9 是否也相對的發生改變，進一步找出廣東住血線蟲感染時導致嗜伊紅性腦膜炎之致病機轉及治療機制，由此模式提供臨床上治療此症之參考。

材料與方法

一. 廣東住血線蟲第三期幼蟲(L_3)之收集:

廣東住血線蟲L_3的收集與鑑定方法依照本實驗室先前的研究成果(Chen et al., 2004)。簡述如下，由田野撿拾非洲大蝸牛

(*Achatina fulica*)，將非洲大蝸牛外殼碾碎，取其組織，剁碎。用組織均質器絞碎，以 1：30 (組織：消化液)比例加入人工胃蛋白酵素消化液(pepsin, Sigma, USA)，以磁性攪拌子於 37℃之恆溫箱中，均勻攪拌消化 2 小時。以雙層紗布濾去雜質，加入生理食鹽水稀釋並靜置，每隔 30 分鐘倒去約一半的上清液，再加入生理食鹽水稀釋靜置，重複上述步驟至完全清澈為止。以滴管吸取下層之沈澱物，置於玻璃皿中，在解剖顯微鏡下，觀察並吸取L_3幼蟲。每 60 隻L_3幼蟲為一單位，置於玻璃培養皿中。

二、體內動物試驗

BALB/c 品系小白鼠(mice)，購自國科會動物中心，為五週齡雄性小白鼠。感染前至少飼養於 12 小時亮及 12 小時暗的動物飼養中心一週。將 90 隻小白鼠隨機分為 5 組，分別為未感染的對照組、廣東住血線蟲感染第 5 天、第 10 天、第 15 天、第 20 天、第 25 天。小白鼠在感染前 12 小時均給予禁水、禁食，每隻小白鼠以口胃管分別灌入 60 隻L_3，於感染後 12 小時再恢復其供水、供食。對照組則灌入等量的生理食鹽水。

四、細胞激素的分析

上述由動物感染採集的 CSF 及細胞株與蟲體共同培養之培養液在 4℃以 12,000 *g* 離心 10 分鐘後，分離上清液於-70℃超低溫冷凍櫃儲存備用。檢體擬測 IFN-γ、IL-5、TNF-α 及 IL-1β 等項目，濃度之檢測採用市售組裝試劑，依照使用說明步驟操作。

五、Zymography

血纖維蛋白酶原活化因子及基質金屬蛋白酶以zymography方法來分析，依照本實驗室先前的研究成果(Chen et al., 2004; Hou et al., 2004)。簡述如下，膠體的配製法(gel preparation) 與SDS-PAGE相同，不同的是separating gel中加入 0.1 % 的gelatin 或 0.1 % 的casein (Sigma, USA)。電泳後，取下gel，加入 100 ml washing buffer(2.5% Triton X-100 in double-distilled

H_2O)清洗gel。在室溫下搖動 30 分鐘，換 washing buffer再洗一次。倒掉washing buffer，gel以double-distilled H_2O 清洗一次。加入 200 ml reaction buffer (40 mM Tris-HCl, pH 8.0, 10 mM $CaCl_2$, 0.01% NaN_3)，在 37℃作用 18 小時以上。以stain solution (0.25% Coomassie Blue R250, in 50% MeOH, 10% acetic acid)染gel 1 小時。以destain solution (20% methanol, 10% acetic acid)退去染液，取膠片以密度掃描儀量化分析結果。

結果

小白鼠 CSF eosinophils 的改變

在感染廣東住血線蟲的小白鼠的 CSF 中，eosinophils 明顯地增加。感染廣東住血線蟲第5天及感染第10天開始治療的組別，用 albendazole 單獨治療或 albendazole-thalidomide 混合治療後，eosinophils 有明顯的減少(P<0.05)，在單獨使用 thalidomide 治療後呈現中等程度的減少。在感染第 15 天開始治療的組別，只有 albendazole-thalidomide 混合治療有明顯減少 eosinophils，其它單獨治療的組別對 eosinophils 的影響則較不顯著 (Figure 1)。

小白鼠 TNF-α 濃度的改變

細胞激素TNF-α在感染廣東住血線蟲的 BLAB/c 小白鼠的 CSF 中明顯的增加。感染廣東住血線蟲第 5 天、第 10 天及第 15 天開始治療的組別，用 albendazole 單獨治療、thalidomode 單獨治療或 albendazole-thalidomide 混合治療後，TNF-α 的濃度皆明顯地下降(P<0.05) (Figure 2)

小白鼠 IL-1β 濃度的改變

細胞激素 IL-1β 在感染廣東住血線蟲的 BLAB/c 小白鼠的 CSF 中明顯的增加。感染廣東住血線蟲第 5 天、第 10 天及第 15 天開始治療的組別，用 albendazole 單獨治療或 albendazole-thalidomide 混合治療後，IL-1β 的濃度明顯地下降(P<0.05)，在單獨使用 thalidomide 則沒有明顯

(P>0.05)的差異(Figure 3)

小白鼠 IL-5 濃度的改變

細胞激素 IL-5 在感染廣東住血線蟲的 BLAB/c 小白鼠的 CSF 中明顯的增加。感染廣東住血線蟲第 5 天開始治療的組別，用 albendazole 單獨治療或 albendazole-thalidomide 混合治療後，IL-5 的濃度明顯地下降(P<0.05)，在單獨使用 thalidomide 則是中等程度地下降。在感染第 10 天或第 15 天開始治療的組別，用 albendazole 單獨治療或 albendazole-thalidomide 混合治療後，IL-5 明顯地下降，而在單獨使用 thalidomode 治療之組別則沒有明顯(P>0.05)的差異(Figure 4)

小白鼠 PAs 活性的改變

Casein zymography 技術可以用來分析 tPA 和 uPA 的活性，tPA 在分子量 70 kDa 的位置可以檢測到，而在感染廣東住血線蟲的 BLAB/c 小白鼠的 CSF 中有明顯的增加。感染廣東住血線蟲第 5 天及感染第 10 天開始治療的組別，用 albendazole 單獨治療或 albendazole-thalidomide 混合治療後，tPA 及 uPA 的活性明顯地下降(P<0.05)，在單獨使用 thalidomide 則是中等程度地下降。在感染第 15 天開始治療的組別，用 albendazole 單獨治療或 albendazole-thalidomide 混合治療後，tPA 及 uPA 則是中度地下降(P<0.05)，而在單獨使用 thalidomode 治療之組別則沒有明顯(P>0.05)的差異(Figure 5 and 6)。

小白鼠 MMP-9 活性的改變

Glatin zymorgraphy 技術可以用來分析 gelatinases 的活性，例如 MMP-9 的酵素活性，MMP-9 在分子量 94 kDa 可以檢測到，此基質金屬蛋白酶在感染廣東住血線蟲的 BALB/c 小白鼠的 CSF 中呈現明顯地(P<0.05)增加。感染廣東住血線蟲第 5 天開始治療的組別，用 albendazole 單獨治療或 albendazole-thalidomide 混合治療後，MMP-9 的活性明顯的降低(P<0.05)。在單獨使用 thalidomide 治療則顯示中等程度的抑制作用。在感染第 10 天或第 15 天開

始治療的組別，用 albendazole 單獨治療或 albendazole-thalidomide 混合治療後，MMP-9 活性明顯地減少($P<0.05$)，而在單獨用 thalidomode 治療的組別則沒有明顯地($P>0.05$)差異(Figure 7)。

討論

絲胺酸家族蛋白酶活性的增加與發炎性疾病(Tarlton *et al*., 2000)、腦膜炎(Winkler *et al*., 2002)有關，而腦膜炎可能因病毒、細菌或其它少見的致病原，如立克次菌、黴菌或寄生蟲(Zhang and Tuomanen, 1999；Casadevall and Pirofski, 2000)所造成。廣東住血線蟲引起的腦膜炎是一種慢性疾病，其特徵為 eosinophil 聚集於蜘蛛膜下腔(Reid and Wallis, 1984)。本研究顯示 tPA 和 uPA 在寄生蟲性腦膜炎中扮演病理性的角色；PAs 會造成細胞外質分解，而其活性的增加和 CSF 中嗜伊紅性白血球的數量有很高的相關性。本研究中 tPA 及 uPA 蛋白酶是由 casein/plasminogen zymography 分析而得知，casein/plasminogen zymography 是以常見的蛋白酶受質酪蛋白(casein)為受質，而以血纖維蛋白酶原做為血纖維蛋白酶(plasmin)受質，而血纖維蛋白酶是經由 PAs 將未活化的 proenzyme 血纖維蛋白酶原活化而得，此試驗方法是以間接方式檢測小白鼠 CSF 中 PAs 的活性。

蛋白分解酵素 MMP-9 活性增高與 blood-CNS barrier 的破壞、血液衍生的免疫細胞(blood-derived immune cells)侵犯神經組織、剝落細胞激素、細胞激素接受器(cytokine receptors)及直接造成周邊與中樞神經系統的細胞性傷害有關(Leppert *et al*., 2001)。BBB 的破壞在細菌性腦膜炎中被視為重要的病理生理現象，神經毒性因子導致腦水腫使腦內的壓力上升(Leib and Täuber, 1999)。在腦部受創的小白鼠中，顯示 uPA 的缺乏會減少蛋白質滲入 CNS 中，可能因 uPA 造成 BBB 破壞 (Kataoka *et al*., 2000)。在小白鼠、天竺鼠(guinea-pigs)、人類等非適當宿主(non-permissive hosts)，遭受廣東住血線蟲

感染時，感染性幼蟲移行至腦部、脊髓或眼睛，導致嚴重的臨床症狀。其最主要的特徵就是於感染後 10 天誘發全身性及腦脊髓液之嗜伊紅性白血球增多，於感染後 20 天達到高原期。因此嗜伊紅性白血球增多一般認為除了具有殺死顱內腔的蟲體外，也是引發中樞神經系統損傷之主因。本研究以廣東住血線蟲感染小白鼠，利用 gelatin substrate zymography 分析腦部 MMP-9 的活性，結果此酵素活性於感染後 10 天明顯增加。另一方面於腦脊髓液之嗜伊紅性白血球數亦在感染後 10 天顯著增加。由此推測 MMP-9 活性增加可能與 blood-CNS barrier 被破壞有關，促進白血球外滲移行至感染的部位，造成發炎反應。albendazole-thalidomide 混合治療後，MMP-9 與腦脊髓液嗜伊紅性白血球的減少呈現正相關。

Blood-CNS barrier 的破壞被認為是感染腦膜炎的重要病理及生理現象，它使得 leuckocytes 可以滲入蜘蛛膜下腔，而 PAs 的蛋白活性及 MMP-9 可以導致 blood-CNS barrier 的分解，而 MMPs 分解酵素可以使 BBB 內皮細胞的 tight junctions 分解，使白血球滲出(Paul et al., 1998; Leppert et al., 2000)。檢測 CSF 的蛋白濃度的方法是一種非侵入性的可靠檢查方法，可用來觀察血液到 CNS 的障壁。Yii (1976)的研究報告顯示廣東住血線蟲所引起的嗜伊紅性腦膜炎的病人比一般人的 CSF 蛋白濃度來得高，而 Romanic and Madri (1994)的研究亦顯示 MMPs 可能藉由破壞血管基底膜的方式來破壞 BBB。本研究結果顯示 albendazole-thalidomide 混合治療明顯地減少 CSF 嗜伊紅性白血球數目，然而，如果單獨使用 thalidomide 治療則只有部分減少。因此，本研究之治療效果可能是藉由減少蛋白分解酵素的活性及降低 CSF 的嗜伊紅性白血球數目，減少 blood-CNS barrier 的破壞。

本研究經過 10 個月的努力，目前完成的動物體內試驗包括廣東住血線蟲感染引發動物之腦膜炎與細胞激素 TNF-α、IL-1β 及 IL-5 的關係；腦膜炎與蛋白分解酵素

tPA、uPA 及 MMP-9 的關係。另外，albendazole-thalidomide 混合治療對細胞激素 TNF-α、IL-1β 及 IL-5 的影響；治療對蛋白分解酵素 tPA、uPA 及 MMP-9 的影響亦已完成。

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圖表

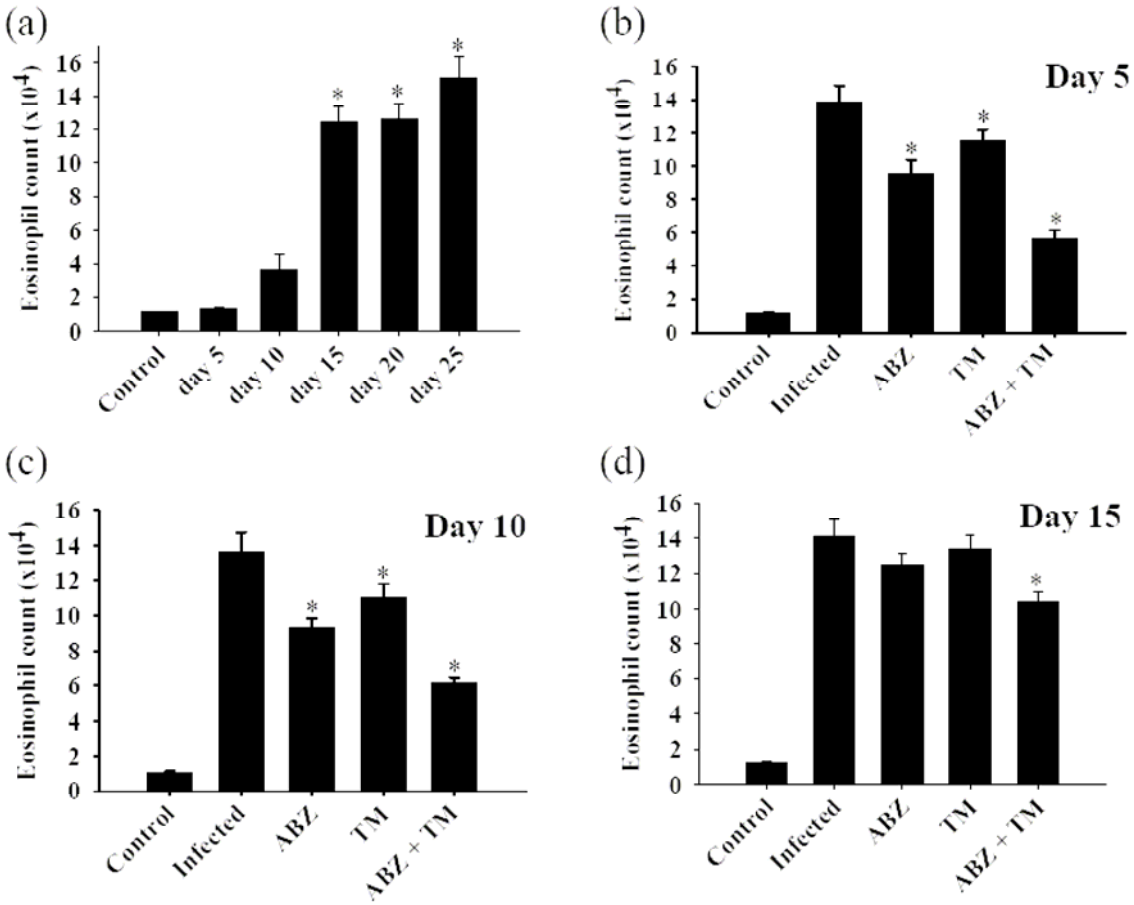

Figure 1. Changes on eosinophil counts. (a) The CSF eosinophils were significantly increased (**P*<0.05) in CSF of mice infected with *Angiostrongylus cantonensis* compared with uninfected control. (b) Treatment on day 5 PI, eosinophils were significantly reduced (**P*<0.05) by albendazole (ABZ) alone, thalidomide (TM) alone or albendazole-thalidomide (ABZ-TM) co-therapy. (c) Treatment on day 10 PI, eosinophils were also significantly reduced (**P*<0.05) after treatment. (d) Treatment on day 15 PI, leukocyte were mildly reduced (**P*<0.05) by albendazole-thalidomide co-therapy, there was no significant difference (*P*>0.05) by albendazole alone or thalidomide alone.

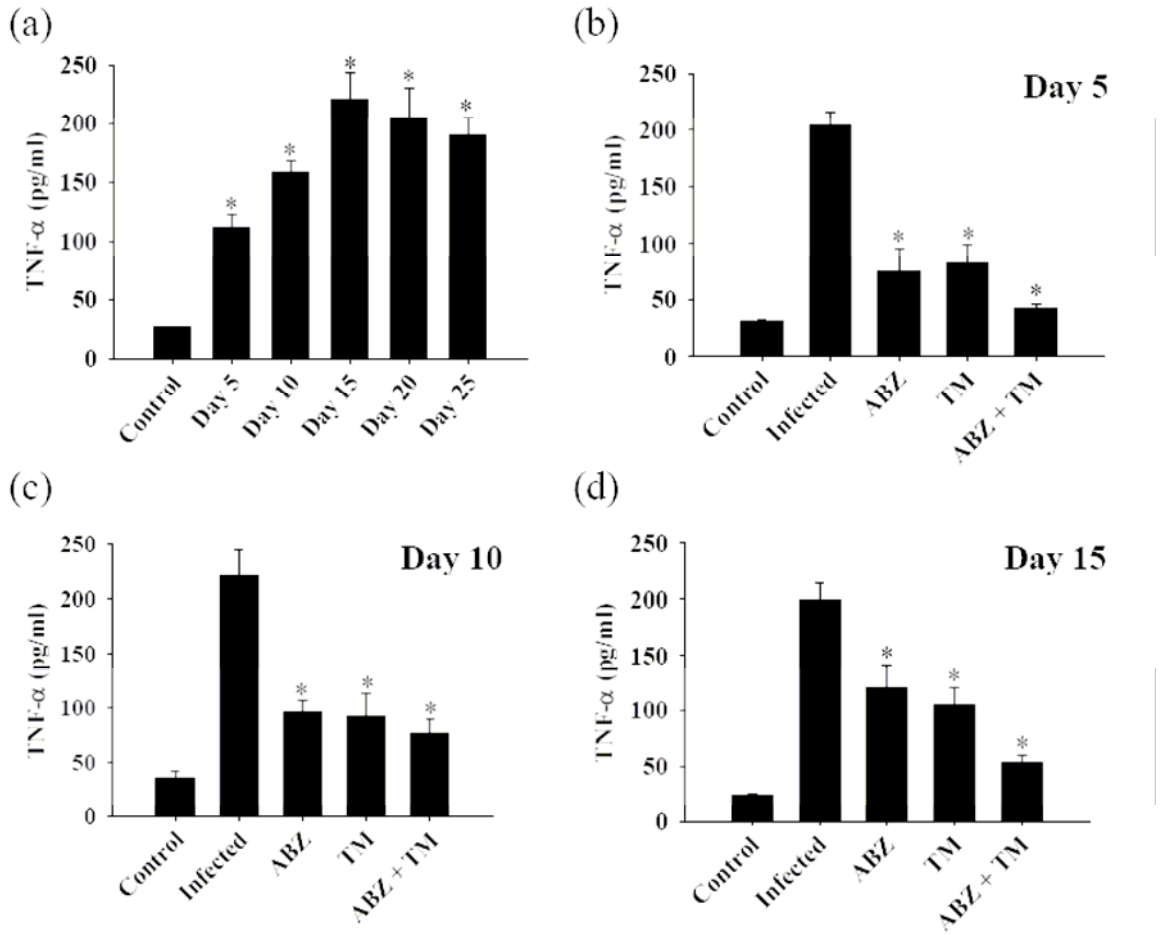

Figure 2. Changes on tumor necrosis alpha (TNF-α) concentrations. (a) *Angiostrongylus cantonensis*-infected mice leads to a significant increase (**P*<0.05) in TNF-α concentrations compared with uninfected control. (b) Treatment on day 5 PI, the TNF-α concentrations were significantly lowered (**P*<0.05) by albendazole (ABZ) alone, thalidomide (TM) alone or albendazole-thalidomide (ABZ-TM) co-therapy compared with infected-untreated mice. (c) Treatment on day 10 PI, the TNF-α concentrations were significantly lowered (**P*<0.05) after treatment compared with infected-untreated mice. (d) Treatment on day 15 PI, the TNF-α concentrations were also significantly lowered (**P*<0.05) after treatment compared with infected-untreated mice.

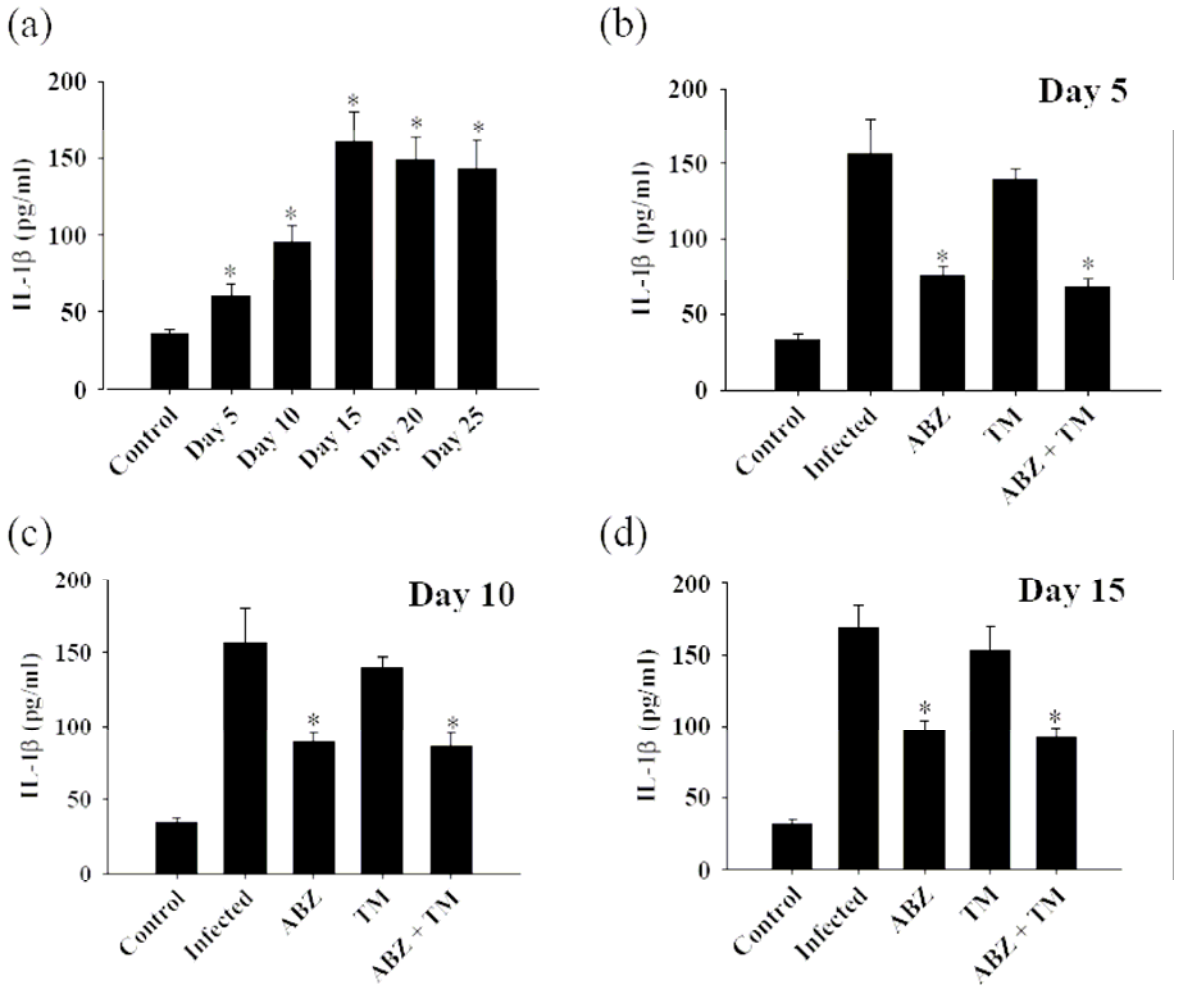

Figure 3. Changes on interleukin (IL)-1β concentrations. (a) *Angiostrongylus cantonensis*-infected mice leads to a significant increase (**P*<0.05) in IL-1β compared with uninfected control. (b) Treatment on day 5 PI, the IL-1β concentrations were significantly lowered (**P*<0.05) by albendazole (ABZ) alone or albendazole-thalidomide (ABZ-TM) co-therapy compared with infected-untreated mice. There was no significant difference (*P*>0.05) by thalidomide (TM) alone. (c) Treatment on day 10 PI, the IL-1β concentrations were significantly lowered (**P*<0.05) by albendazole alone or albendazole-thalidomide co-therapy compared with infected-untreated mice. (d) Treatment on day 15 PI, the IL-1β concentrations were significantly lowered (**P*<0.05) by albendazole alone or albendazole-thalidomide co-therapy compared with infected-untreated mice.

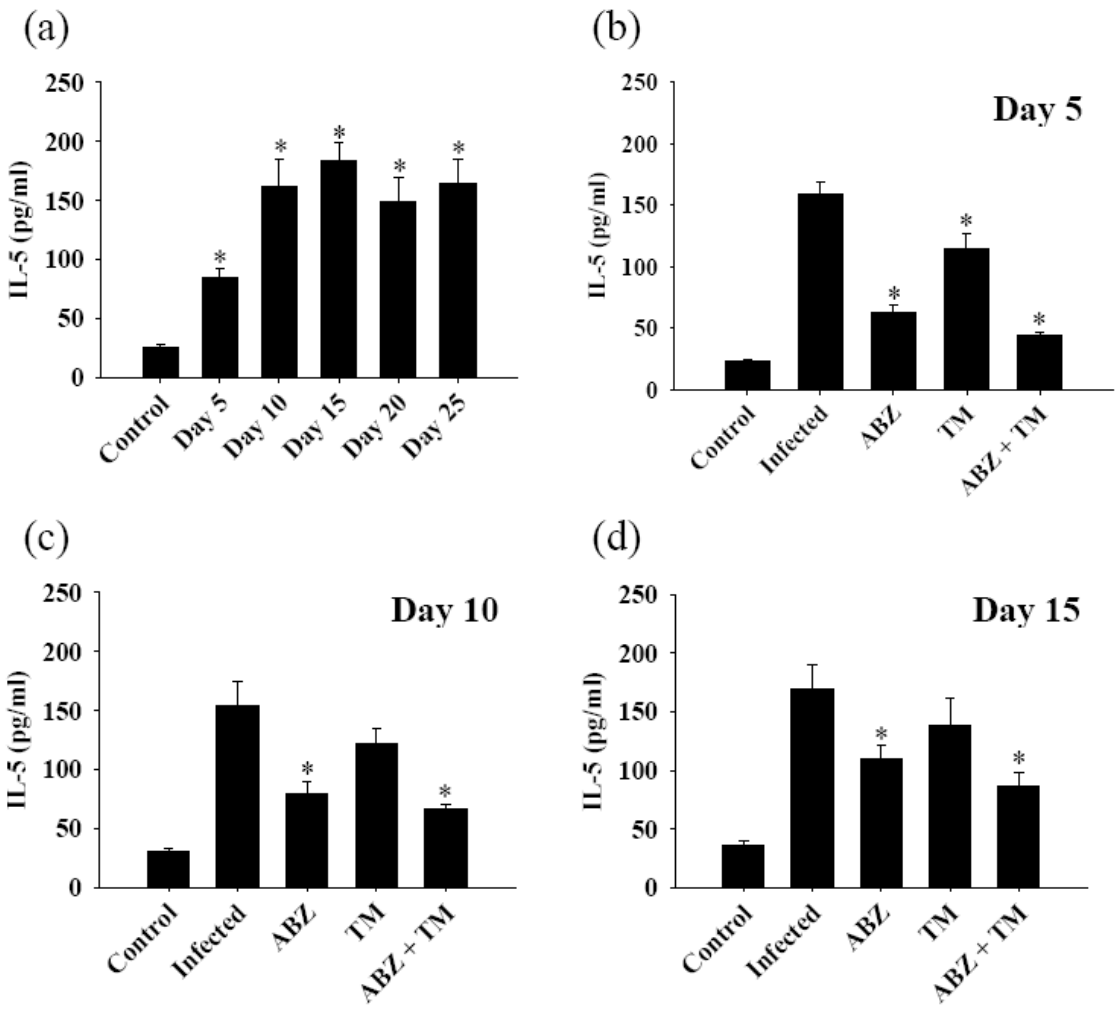

Figure 4. Changes on interleukin (IL)-5 concentrations. (a) *Angiostrongylus cantonensis*-infected mice leads to a significant increase (**P*<0.05) in IL-5 compared with uninfected control. (b) Treatment on day 5 PI, the IL-5 concentrations were significantly lowered (**P*<0.05) by albendazole (ABZ) alone, thalidomide (TM) alone or albendazole-thalidomide (ABZ-TM) co-therapy compared with infected-untreated mice. (c) Treatment on day 10 PI, the IL-5 concentrations were significantly lowered (**P*<0.05) by albendazole alone or albendazole-thalidomide co-therapy compared with infected-untreated mice. There was no significant difference (*P*>0.05) by thalidomide alone. (d) Treatment on day 15 PI, the IL-5 concentrations were significantly lowered (**P*<0.05) by albendazole alone or albendazole-thalidomide co-therapy compared with infected-untreated mice.

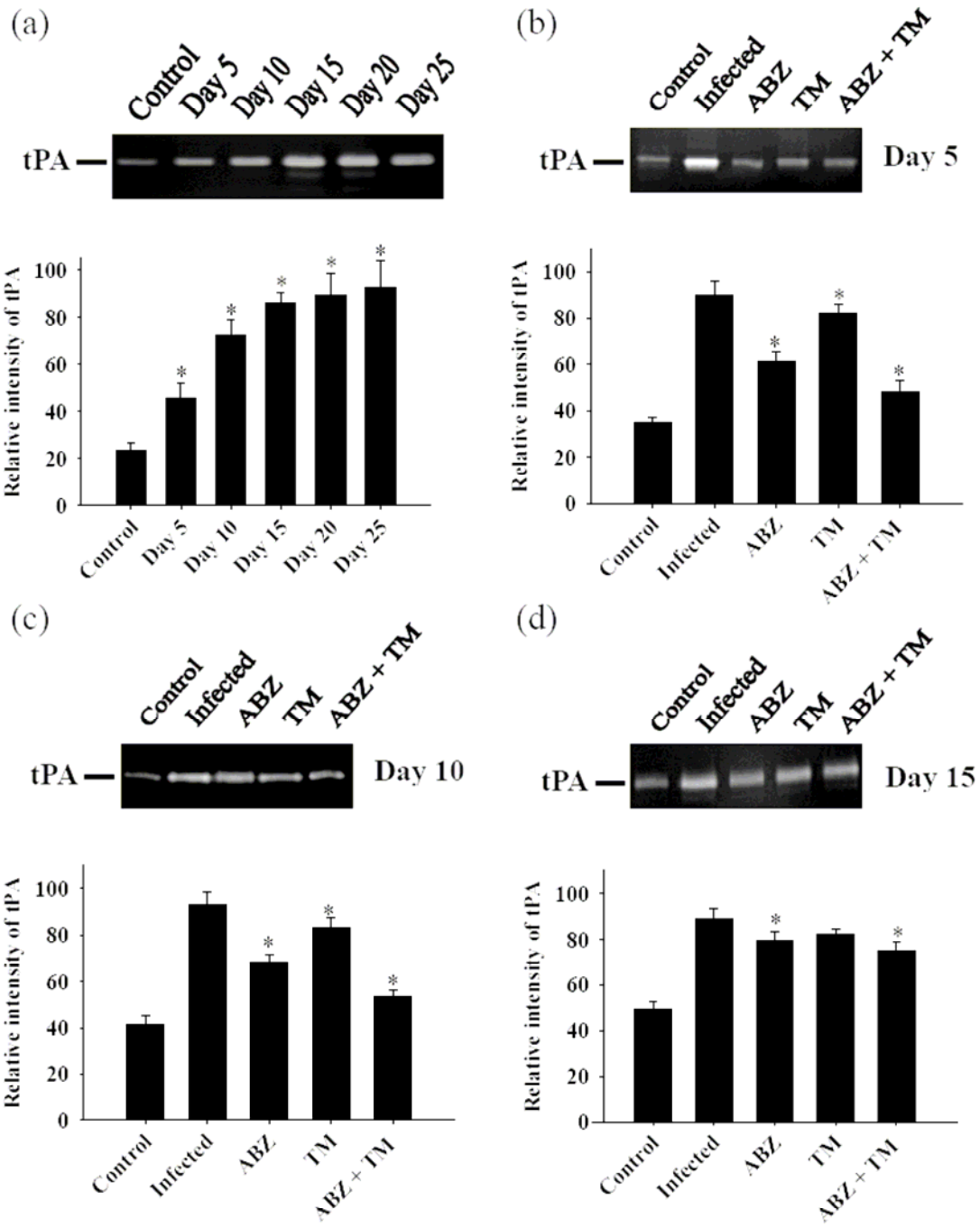

Figure 5. Changes on tissue-type plasminogen activator (tPA) activity. (a) The tPA was significantly increased (**P*<0.05) in CSF of mice infected with *Angiostrongylus cantonensis* compared with uninfected control. (b) Treatment on day 5 PI, the activities of tPA were significantly reduced (**P*<0.05) by albendazole (ABZ) alone, thalidomide (TM) alone or albendazole-thalidomide (ABZ+ TM) co-therapy. (c) Treatment on day 10 PI, the activities of tPA were also significantly reduced (**P*<0.05) after treatment. (d) Treatment on day 15 PI, the activities of tPA were mildly reduced (**P*<0.05) by albendazole alone or albendazole-thalidomide co-therapy, whereas no significant changed (*P*>0.05) in treatment with thalidomide alone. Quantitative analysis of the tPA activity was performed with a computer-assisted imaging densitometer system.

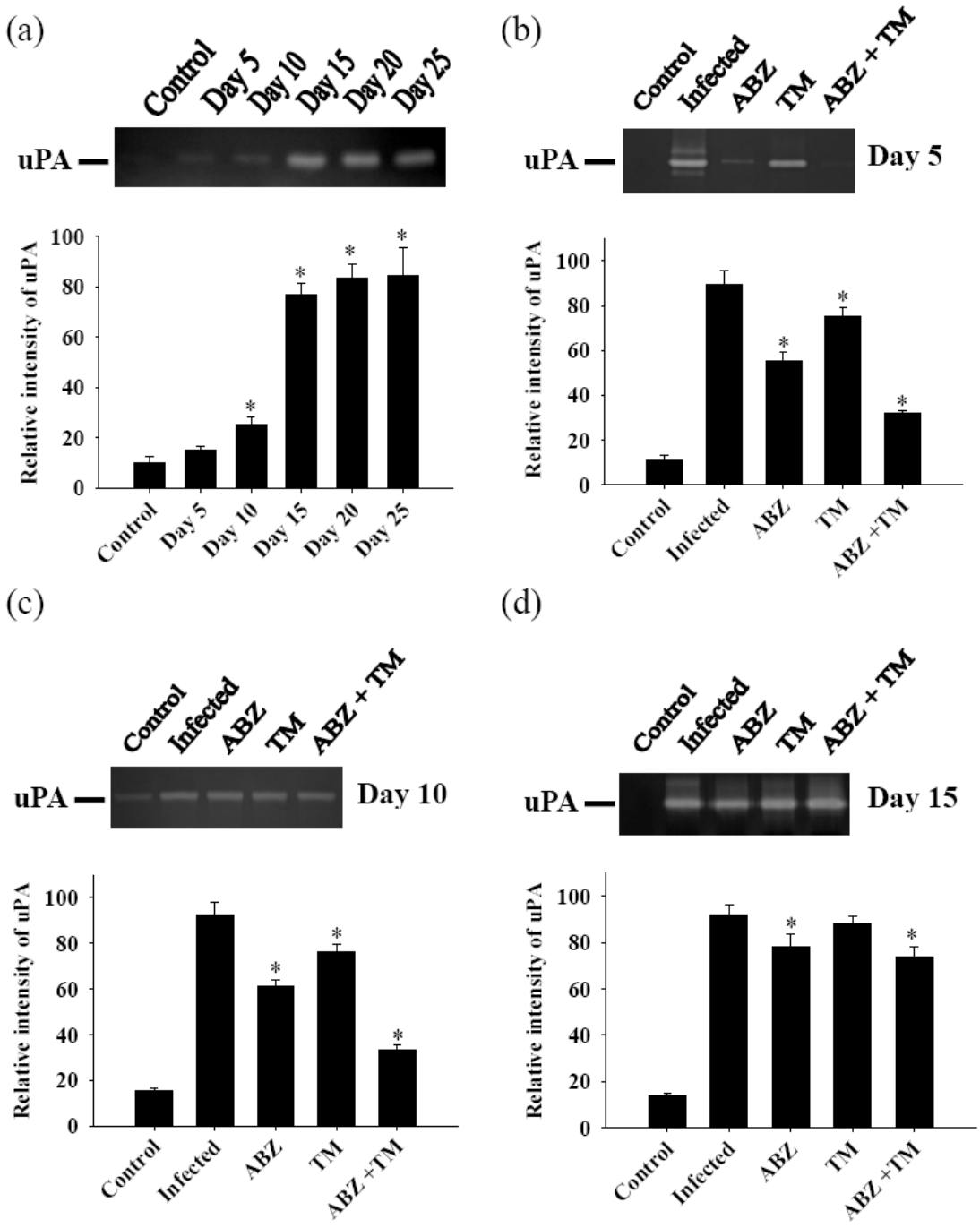

Figure 6. Changes on urokinase-type plasminogen activator (uPA) activity. (a) The uPA was significantly increased (*P<0.05) in CSF of mice infected with *Angiostrongylus cantonensis* compared with uninfected control. (b) Treatment on day 5 PI, the activities of uPA were significantly reduced (*P<0.05) by albendazole (ABZ) alone, thalidomide (TM) alone or albendazole-thalidomide (ABZ-TM) co-therapy. (c) Treatment on day 10 PI, the activities of uPA were also significantly reduced (*P<0.05) after treatment. (d) Treatment on day 15 PI, the activities of uPA were mildly reduced (*P<0.05) by albendazole alone or albendazole-thalidomide co-therapy, whereas no significant changed (P>0.05) by thalidomide alone. Quantitative analysis of the uPA activity was performed with a computer-assisted imaging densitometer system.

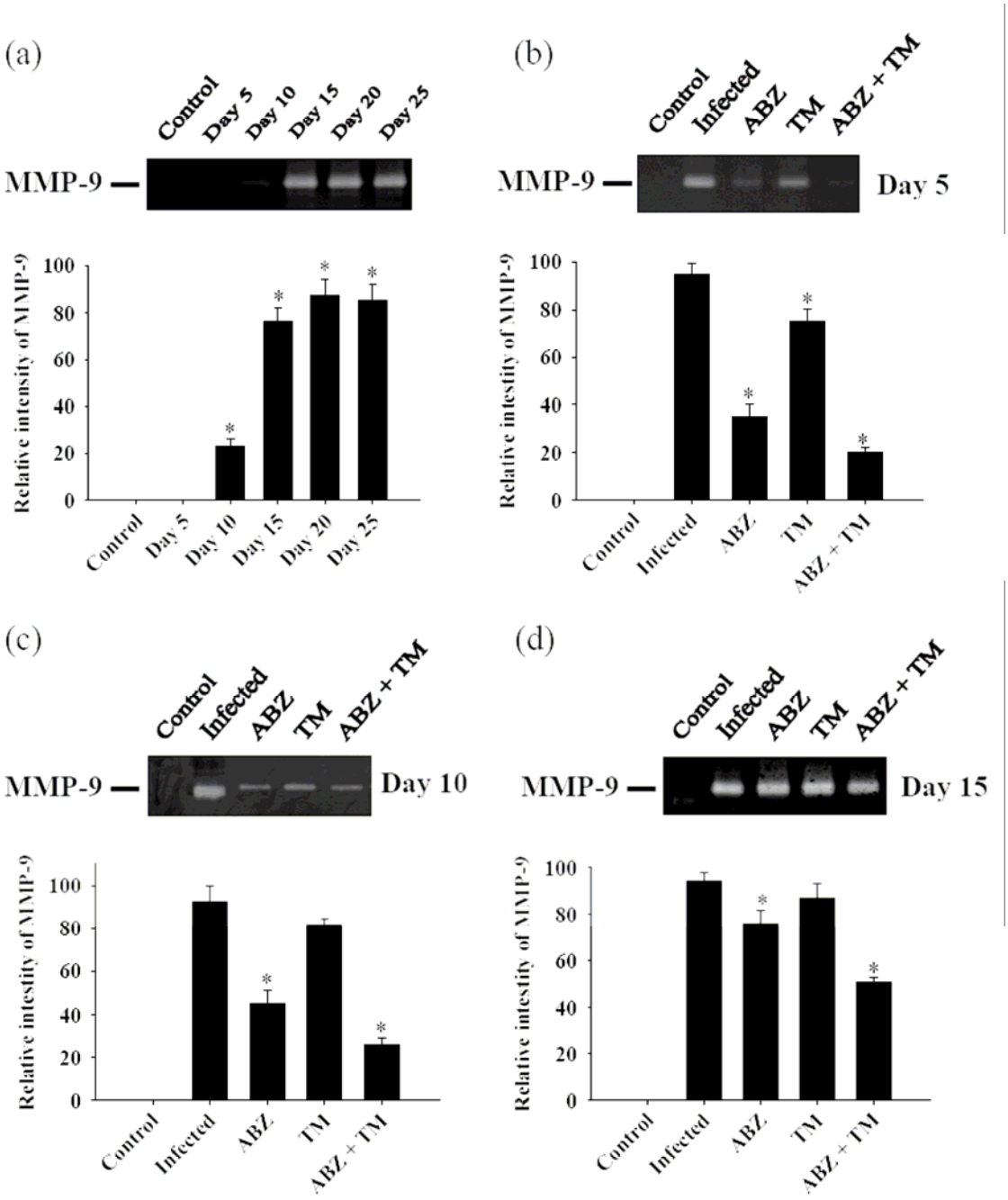

Figure 7. Changes on matrix metalloproteinase-9 (MMP-9) activity. (a) The MMP-9 was significantly increased (**P*<0.05) in CSF of mice infected with *Angiostrongylus cantonensis* compared with uninfected control. (b) Treatment on day 5 PI, the activities of MMP-9 were significantly reduced (**P*<0.05) by albendazole (ABZ) alone, thalidomide (TM) alone or albendazole-thalidomide (ABZ-TM) co-therapy. (c) Treatment on day 10 PI, the activities of MMP-9 were significantly reduced (**P*<0.05) by the individual use of albendazole or albendazole-thalidomide co-therapy. There was no significantly difference by thalidomide alone. (d) Treatment on day 15 PI, the activities of MMP-9 were mildly reduced (**P*<0.05) in albendazole alone or albendazole-thalidomide co-therapy, whereas no significant changed (*P*>0.05) in treatment with thalidomide alone. Quantitative analysis of the MMP-9 activity was performed with a computer-assisted imaging densitometer system.

計劃成果自評

一、 研究內容與原計畫相符程度達 80%

二、 達成預期目標情況：Albendazole-thalidomide 混合治療對 IL-1β、IL-5、tPA、uPA、MMP-9、對蜘蛛膜下腔發炎反應的影響。Albendazole-thalidomide 混合治療對的影響。

三、 研究成果之學術或應用價值：藉由廣東住血線蟲感染誘發與人類模式相似之動物腦膜炎，瞭解 albendazole-thalidomide 混合治療對細胞激素、血纖維蛋白酶原活化因子及基質金屬蛋白酶之影響。

四、 適合在學術期刊發表

五、 主要發現：廣東住血線蟲症的治療早期療效較佳，在同樣的治療劑量及感染時間，較早的治療顯示較佳的結果，這種使用 albendazole-thalidomide 混合治療的有效方法可提供治療寄生蟲性腦膜炎的新方法。