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評估 thalidomide 及 albendazole 混合治療廣東住血線蟲感染 BALB/c 小白鼠所引發腦膜炎之效果

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評估 thalidomide 及 albendazole 混合治療廣東住血線蟲感染 BALB/c 小白鼠所引發寄生蟲性腦膜炎之效果

Efficacy of thalidomide–albendazole co-therapy against *Angiostrongylus cantonensis* induced parasitic meningitis in BALB/c mice

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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 (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，但是死亡的幼蟲卻會造成腦部的傷害，並引起更嚴重的免疫反應。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。本研究使用 albendazole 和 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)蟲的回收數。(2) 組織病理的改變。 (3) eosinophil 的計數。(4) 組織型血纖維蛋白酶活化因子(tissue-type plasminogen activator)、尿素型血纖維蛋白酶活化因子(urokinase-type plasminogen activator)、基質金屬蛋白酶-9 (matrix metalloproteinase-9)及 albumin。本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，顯示治療的時間點很重要且其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能提供了一個對於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. 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) worm recovery; 2) histopathological score of meningitis; 3) eosinophil counts; 4)

tissue-type PA, urokinase-type PA and MMP-9; and 6) albumin. 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

廣東住血線蟲症(angiostrongliasis)是由大白鼠肺部的廣東住血線蟲所引起的，其廣泛地分布在熱帶及亞熱帶地區的嚙齒類動物中，此蟲會引起的中樞神經系統疾病，每年在台灣都有幾個病例報告 (Cheng et al., 1984; Tsai et al., 2004)。本研究室先前的研究顯示組織型血纖維蛋白酶活化因子(tissue-type plasminogen activators, tPA)和尿素型血纖維蛋白酶活化因子(urokinase-type plasminogen activators, uPA)可能和 BALB/c 小白鼠感染廣東住血線蟲引起嗜伊紅性腦膜炎有關(Hou et al., 2004)，另外，本研究室亦證實基質金屬蛋白酶-9 (matrix metalloproteinase-9, MMP-9)也與嗜伊

紅性腦膜炎有關，且在廣東住血線蟲性腦膜炎的 BLAB/c 小白鼠和 ICR 小白鼠中可能是一個重要的感染指標 (Lai et al., 2004; Lee et al., 2004)。血腦障壁(blood-brain barrier, BBB)的破壞是神經發炎的一個重要特徵，與發炎細胞、體液和蛋白質(包含補體及細胞激素)的聚集有關(Rosenberg et al., 1992)。過去的研究顯示 MMP 酵素系統與 BBB 的破壞有關 (Rosenberg et al., 1992; Leppert et al., 2000)。

引起發炎的細胞激素腫瘤壞死因子 α (tumor necrosis factor α, TNF-α)可能扮演 neutrophil 的活化因子，媒介粘附作用(adherence)、趨化作用 (chemotaxis)和去顆粒作用 (degranulation)中重要的活化因子，它被認為是發生在慢性感染中的嚴重惡病質因子(Beutler and Cerami, 1989)，MMP-9 已經顯示其會清除未活化的 TNF-α，使得活化的 TNF-α 得以釋放 (Gearing et al., 1994)，除此之外，MMP-9 基因的表現可藉由 macrophages 和 polymorphonuclear leucocytes，因此 pro-MMP 的釋放是藉由 TNF-α 和 interleukin-1 beta (IL-1β)所引發 (Saren et al., 1996; Pugin et al., 1999)。

一些的驅蟲藥能有效用來治療感染人體的廣東住血線蟲，但是在腦部死亡的蟲體卻可能會引起免疫反應而導致腦部的損傷。Albendazole 一直被認為是治療廣東住血線蟲病的有效藥物，此葯經常與類固醇混合治療以達到避免因蟲體死亡所引起的發炎反應，然而類固醇的治療一直存在著爭議性。因此本研究以驅蟲藥 albendazole 與 TNF-α 抑制劑 thalidomide 混合治

療，評估此療法在廣東住血線蟲所引起寄生蟲性腦膜炎的小白鼠之療效，進一步了解 TNF-α 抑制劑 thalidomide 是否能取代類固醇治療。

材料與方法

實驗動物

由國科會動物中心(National Laboratory Animal Center, Taipei, Taiwan)購買五週大 BALB/c strain 的雄性小白鼠。給予小白鼠 12 小時光照和 12 小時黑暗的光週期環境，並提供 Purina Laboratory Chow 飼料和水，直到感染前送至本實驗室進行試驗。

幼蟲的製備

廣東住血線蟲的第三期幼蟲是由屏東田間取得的非洲大蝸牛(*Achatina fulica*)純化而來。幼蟲的回收是以 Parson 和 Grieve 的方法(1990)稍作修改而成。簡言之，將蝸牛殼壓碎後，所取得的組織經均質化後再加入 pepsin-HCL 溶液(pH 1-2, 500 I.U. pepsin/g tissue)分解，而後在 37℃的水浴槽中作用 2 小時。將前述溶液離心 1400 *g*, 10 分鐘後倒掉上清液。取沉澱物於顯微鏡下觀察幼蟲。而廣東住血線蟲第三期幼蟲的型態鑑定為蟲體長 425~524 μm，寬 23~34 μm。其尾端部分為細長(Ash, 1970)。為進一步確認所取得的幼蟲為廣東住血線蟲，將幼蟲餵食大白鼠，於 2 至 3 週後取其腦部組織觀察是否有此蟲感染。

動物感染

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 50 隻第三期幼蟲，於感染後 12 小時再恢復其供水、供食。

藥物

Thalidomide (THADO capsule) 每顆膠囊含主成分thalidomide 50 mg，化學名α-(N-phthalimido) glutarimide，它是glutamic acid的衍生物，外觀是白色結晶，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，是一種免疫調節劑(immuno-modulatory agent)。

治療

將感染廣東住血線蟲的動物隨機分五組，未治療之對照組包括感染及未感染廣東住血線蟲兩組，實驗組分為三組，分別在感染後第 5 天、10 天及 15 天，以口胃管投與 albendazole、thalidomide 及 albendazole 與 thalidomide 混合治療，albendazole 劑量為每公斤體重 5、10 或 20 毫克，thalidomide 劑量為每公斤體重 25、50 或 100 毫克，連續投藥 7 天，在第 22 天將動物犧牲。而對照組則灌食生理食鹽水，在犧牲實驗組動物時也同時犧牲對照組動物做比較。

病理檢查

動物犧牲時將腦蓋剝開，評估其腦膜出血情形，取出之腦置於培養皿中，將腦膜剝離，以生理食鹽水沖洗，收集此液作為腦脊髓液，分析其中嗜伊紅性白血球數目、tPA 活性、uPA 活性及 MMP-9 活性，接著以解剖顯微鏡將蟲體挑出，計算其數目。

組織學技術 (H&E staining)

小白鼠腦部組織的處理方法依照本實驗室的研究成果(Lai et al., 2004)。簡述如下，組織以 formol-alcohol (formaline : acetic acid : 70% alcohol = 1 : 1 : 20) 固定 24 小時，以序列酒精(50%, 70%, 80%, 90%, 100%)脫水，以二甲苯(xylene)置換酒精，最後以石蠟(paraffin)包埋。切片以 haematoxylin (Muto, Japan)染色 5 分鐘、1% eosin Y solution (Muto, Japan) 染色 2 分鐘，在染色完成之玻片上滴上 mounting media，在光學顯微鏡下觀察並拍照。

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

治療對蟲回收數的影響

感染廣東住血線蟲第 5 天及感染第 10 天開始治療的組別，albendazole 單獨治療和用 albendazole-thalidomide 混合治療的小白鼠腦組織所回收的蟲數明顯減少(P<0.05)，而在沒有治療或 thalidomide 單獨治療的蟲回收數則沒有明顯的差異(P>0.05)。感染 15 天開始治療的組別在使用 albendazole 單獨治療或 albendazole-thalidomide 混合治療與未治療的情形比較下則是有減少的趨勢，然而蟲回收數仍然相當高，殺幼蟲效果明顯比感染 5 天開始治療低 (Table 1-3)。

治療對病理變化的影響

腦組織以 haematoxylin 和 eosion 進行組織染色，光學顯微鏡觀察顯示感染廣東住血線蟲的 BLAB/c 小白鼠會引起嗜伊紅性腦膜炎。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，leukocytes 有明顯地減少(P<0.05)，但是單獨使用 thalidomide 治療則只有中等程度的減少。在感染第 15 天開始治療的組別，用 albendazole 單獨治療或是用 albendazole-thalidomide 混合治療後，leukocytes 則是呈現中等程度的減少，在單獨使用 thalidomide 治療與感染未治療的控制組之間並沒有明顯地差異。而幼蟲在使用 albendazole 單獨治療或 albendazole-thalidomide 混合治療後有退化(degenation)的現象(Fig. 1)。

治療對 CSF eosinophils 的影響

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，使用 albendazole 單獨治療或 albendazole-thalidomide 混合治療後，eosinophils 則是有明顯的減少 ($P<0.05$)，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，治療後對 eosinophils 的影響則較小 (Fig. 2)。

治療對 PAs 活性的影響

Casein zymography 技術可以用來分析 tPA 和 uPA 的活性，tPA 在分子量 70 kDa 附近可以檢測到，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 則是中度地下降。在感染第 15 天開始治療的組別，用 albendazole-thalidomide 混合治療後，tPA 及 uPA 則是中度地下降，而在單獨使用 albendazole 或 thalidomode 治療之組別則沒有明顯 ($P>0.05$)的差異(Fig. 3 and 4)。

治療對 MMP-9 活性的影響

Glatin zymorgraphy 技術可以用來分析 gelatinases 的活性，例如 MMP-9 的活化蛋白的酵素活性，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，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 15 天開始治療的組別，用 albendazole-thalidomide 混合治療後，MMP-9 活性中等程度的，而在單獨用 albendazole 或 thalidomode 治療的組別則沒有明顯地(P>0.05)差異(Fig. 5)。

治療對 albumin 的影響

在感染廣東住血線蟲的小白鼠，CSF albumin 明顯地(P<0.05)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-thalidomide 混合治療後，CSF albumin 的濃度明顯(P<0.05)的降低。在單獨用 thalidomide 治療後則是顯現中等程度的降低。在感染第 15 天開始治療的組別，用 albendazole-thalidomide 混合治療後，CSF albumin 則是中等程度的下降，而在單獨使用 albendazole 或 thalidomode 治療之組別則沒有明顯地(P>0.05)差異(Fig. 6)。

討論

Thalidomide 在臨床的作用主要是藉由改變循環免疫細胞的表現及減少 cytokine 的合成 (Nogueira et al., 1994) 和抑制 macrophage 合成 TNF-α (Sampaio et al., 1991)。改變細胞激素的合成和釋放及 lymphokine 的合成是重要的，如同改變 lymphocyte 的運輸及 neutrophil 移行是一樣的。因此，內皮

細胞扮演重要的角色在 leukocyte 的 extravasation 及 maintenance。我們很清楚地了解 thalidomide 誘發細胞吸附因子改變及 leukocytes 和 endothelial cell 之間的作用發生變化，造成發炎及免疫反應的調節 (Meierhofer et al., 2001)。而腦部發炎的程度是受到停留在 CNS 的幼蟲數來決定，因此減少 CNS 的幼蟲數可以減輕腦部的病理現象。另外，廣東住血線蟲在感染 15 天後會在腦部發育為第五期的幼蟲(年輕成蟲)，所以在感染 15 天開始治療比起感染第 5 天開始治療困難，這些數據顯示選擇治療的時間點是很重要的，且療效與驅蟲藥的關係也很密切，這也是本研究選擇在感染廣東住血線蟲後的第 5 天及第 15 天進行治療並在感染 22 天犧牲的主要原因。

我們的研究結果清楚地顯示 albendazole-thalidomide 混合治療明顯抑制 PAs 的活性與發炎，而單獨使用 thalidomide 治療則只能部分抑制 PAs 與發炎反應，此結果顯示一個重要的問題，是否抑制 PAs 與腦膜炎的復原有密切的相關性? 換句話說，就是 PAs 活性減少是腦膜炎復原的現象，令人興奮的是在使用 albendazole-thalidomide 混合治療可以使得 PAs 活性減少且腦膜炎有了明顯的改善，這種新的治療提供了治療寄生蟲性腦膜炎一種新的方法。

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沒有侵入性的可靠檢查方法，可用來來觀察血液到 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)的研究易顯示 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 albumin 的濃度，然而，如果單獨使用 thalidomide 治療則只有部分減少。這些結果顯示 CSF 的蛋白含量可能在嗜伊紅性腦膜炎中扮演了一個重要的指標。因此，治療可能藉由減輕 PAs/MMP-9 的活性及降低 CSF 的蛋白含量，減少 blood-CNS barrier 的破壞。

Thalidomide 抑制 TNF-α 的產生，因此能夠降低發炎反應 (Meierhofer et al., 2001)。在正常生長及發育中，TNF-α 的表現維持基本的 level，本研究結果顯示 TNF-α 在感染廣東住血線蟲時會增加，albendazole-thalidomide 混合治療明顯地降低 TNF-α 及 IL-5 的 mRNA level，然而 mRNA 的 level 沒有完全被消除。Albendazole-thalidomide 混合治療把 cytokines(TNF-α 及 IL-5)作為治療的目標也許提供了一個對於嗜伊紅性腦膜炎有效治療方法。

總結來說，使用 albendazole 與 thalidomide 混合治療罹患廣東住血線蟲症的小白鼠，明顯地減少了幼蟲的回收數、eosinophil 數量、tPA、uPA、MMP-9 和 CSF albumin 等治療指標，這種合併治療可能是抑制 PAs/MMp-9 酵素系統進而改善嗜伊紅性腦膜炎的

協同作用所致。

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圖表

Table. 1 Effect of albendazole on *Angiostrongylus cantonensis* larvae in BALB/c mice treated for 7 days at different dosages and starting medication

Medication			Number of worms recovered Mean±SD	Worm reduction %
Oral dosage (mg/kg/day)	Days post-inoculation	Days treated		
Control (5)	—	—	30.0±3.5	—
5	5	7	6.5±1.8	78.4
10	5	7	3.3±1.9	89
20	5	7	3.6±2.2	88
Control (10)	—	—	31.4±5.1	—
5	10	7	8.9±1.6	71.7
10	10	7	6.2±1.8	80.3
20	10	7	6.7±2.1	80.7
Control (15)	—	—	29.2±3.5	—
5	15	7	16.3±2.1	44.2
10	15	7	13.6±2.6	53.5
20	15	7	11.2±1.9	61.7

Each group consists of 5 mice infected with 50 infective larvae and sacrificed on day 22 post-inoculation.

The infected-untreated control mice (5, 10, 15) were infected with 50 larvae and inoculated with distilled water by oral inoculation on day 5, 10, and 15 PI, respectively.

Table. 2 Effect of thalidomide on *Angiostrongylus cantonensis* larvae in BALB/c mice treated for 7 days at different dosages and starting medication

Medication			Number of worms	Worm reduction
Oral dosage (mg/kg/day)	Days post-inoculation	Days treated	recovered Mean±SD	%
Control (5)	—	—	31.0±3.8	—
25	5	7	28.0±3.6	9.7
50	5	7	27.2±4.1	12.3
100	5	7	26.6±4.2	14.2
Control (10)	—	—	32.0±3.1	—
25	10	7	29.8±3.7	7.9
50	10	7	27.3±3.5	14.7
20	10	7	26.5±4.1	17.2
Control (15)	—	—	28.0±2.8	—
25	15	7	26.4±3.9	5.8
50	15	7	26.2±3.7	6.5
100	15	7	25.8±3.5	7.9

Each group consists of 5 mice infected with 50 infective larvae and sacrificed on day 22 post-inoculation.

The infected-untreated control mice (5, 10, 15) were infected with 50 larvae and inoculated with distilled water by oral inoculation on day 5, 10, and 15 PI, respectively.

Table. 3 Effect of albendazole–thalidomide co-therapy on *Angiostrongylus cantonensis* larvae in BALB/c mice treated for 7 days at different starting medication

Medication		Number of worms recovered	Worm
Days post-incoculation	Days treated	Mean±SD	reduction (%)
Control	—	31.0±4.5	—
5	7	1.2±2.1	96.2
10	7	4.3±1.9	86.2
15	7	11.6±1.8	62.6

Each group consists of 5 mice infected with 50 infective larvae and sacrificed on day 22 post-inoculation.

The albendazole-thalidomide co-therapy mice received a treatment combining albendazole (10 mg/kg/day) and thalidomide (50 mg/kg/day) for 7 consecutive days on day 10 PI.

The infected-untreated control mice were infected with 50 larvae and inoculated with distilled water by oral inoculation on day 10 PI.

Fig. 1

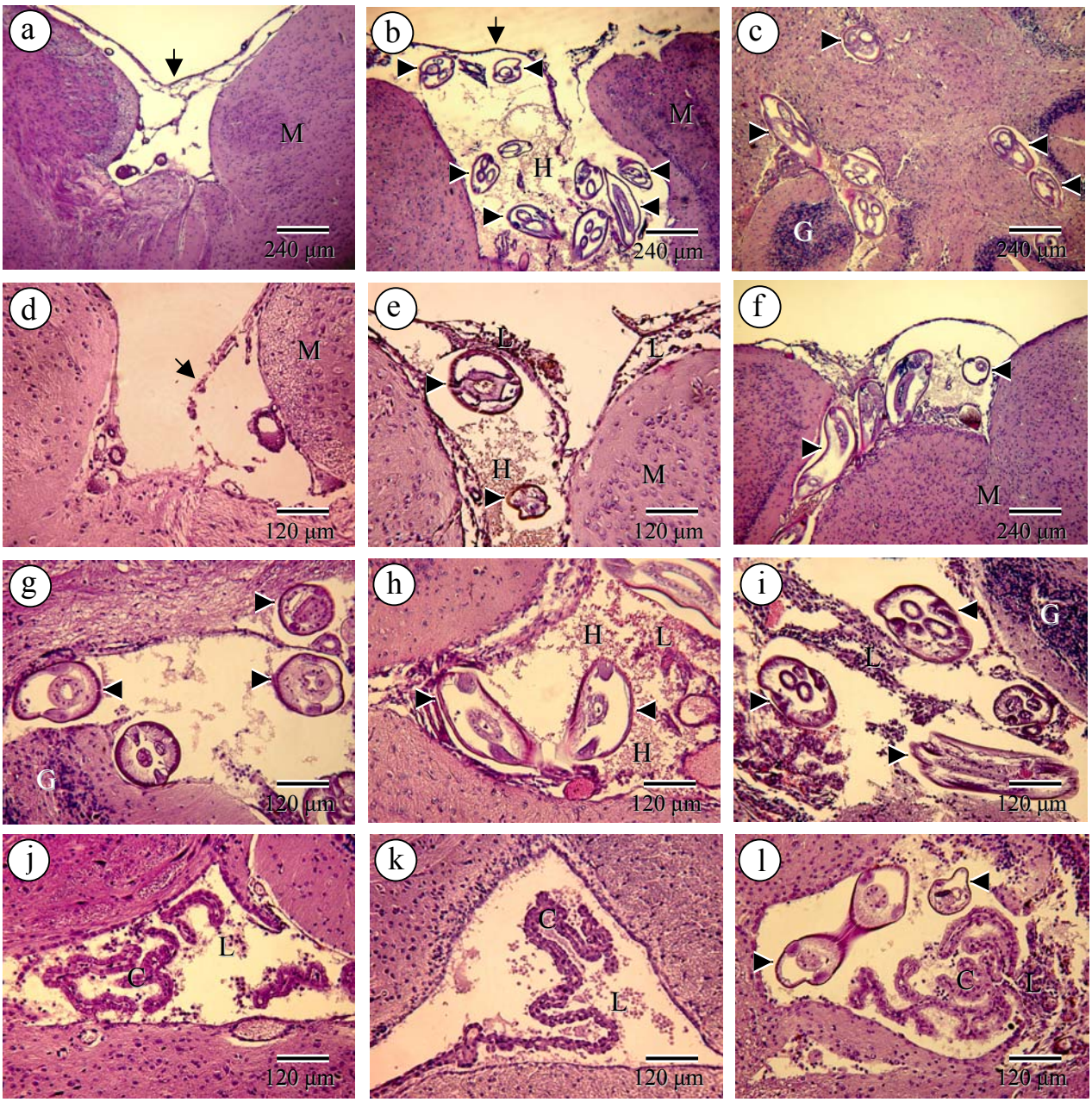

a
M
240 µm
b
M
H
240 µm
c
G
240 µm
d
M
120 µm
e
L
L
H
M
120 µm
f
M
240 µm
g
G
120 µm
h
H
L
H
120 µm
i
G
L
120 µm
j
L
C
120 µm
k
C
L
120 µm
l
C
L
120 µm

FIG. 1. Histological observations. (a) The uninfected control presented normal meninges (arrowhead) and almost no leukocytes in the subarachnoid space. (b) The subarachnoid space of mice infected with *Angiostrongylus cantonensis* showing larvae (arrowheads) and leukocytes (L) accumulation. (c) The ventricle of mice infected with *Angiostrongylus cantonensis* showing larvae (arrowheads) and leukocytes (L) accumulation. (d) Treatment with albendazole on day 5 PI significantly decreased the larvae and leukocyte numbers (L). (e) Treatment with albendazole on day 10 PI significantly decreased the larvae and leukocyte numbers (L). (f) Treatment with albendazole on day 15 PI mildly degenerated the larvae (arrowheads) and mildly decreased the leukocyte numbers (L). (g) Treatment with thalidomide on day 5 PI could not kill the larvae (arrowheads) and only mildly decreased the leukocyte numbers (L). (h) Treatment with thalidomide on day 10 PI could not kill the larvae (arrowheads) and only mildly decreased the leukocyte numbers (L). (i) Treatment with thalidomide on day 15 PI could not decrease the leukocyte numbers (L). (j) Albendazole-thalidomide co-therapy was significantly decreased the leukocyte numbers (L) on day 5 PI. (k) Albendazole-thalidomide co-therapy was significantly decreased the leukocyte numbers (L) on day 10 PI. (l) Co-therapy with albendazole and thalidomide significantly degenerated the larvae (arrowheads) and mildly decreased the leukocyte numbers (L) on day 15 PI.

Fig. 2

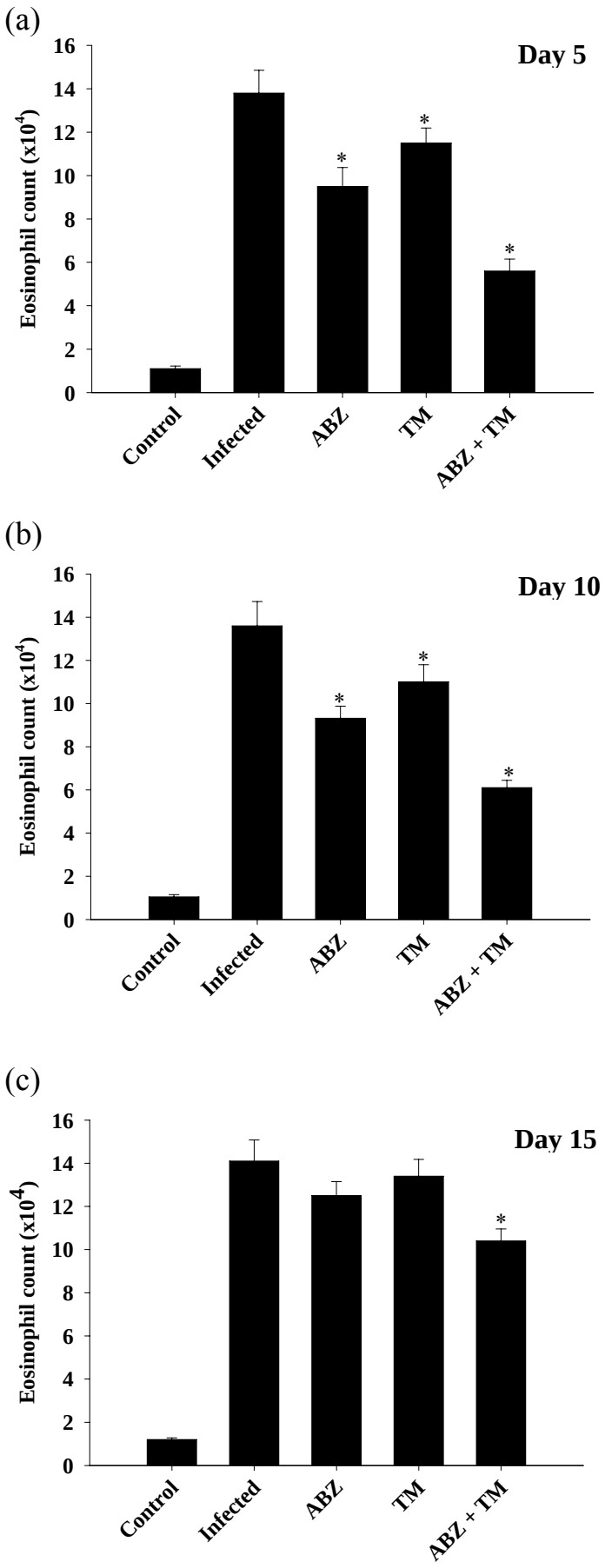
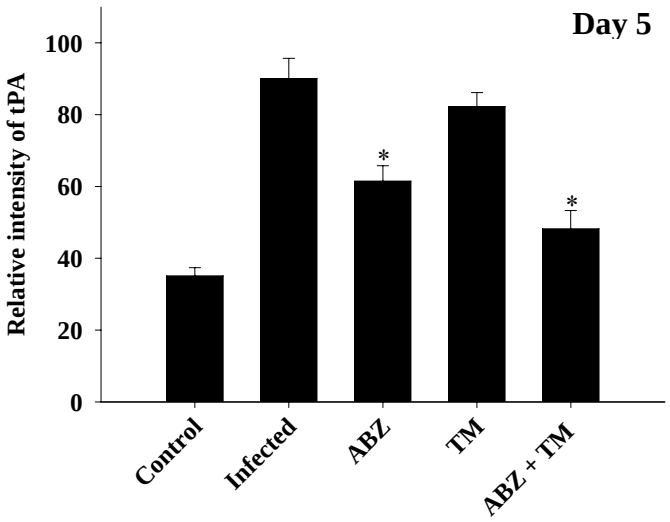

(a)
Day 5
Eosinophil count (x10^4)
16
14
12
10
8
6
4
2
0
*
*
*
Control
Infected
ABZ
TM
ABZ + TM
(b)
Day 10
Eosinophil count (x10^4)
16
14
12
10
8
6
4
2
0
*
*
*
Control
Infected
ABZ
TM
ABZ + TM
(c)
Day 15
Eosinophil count (x10^4)
16
14
12
10
8
6
4
2
0
*
Control
Infected
ABZ
TM
ABZ + TM

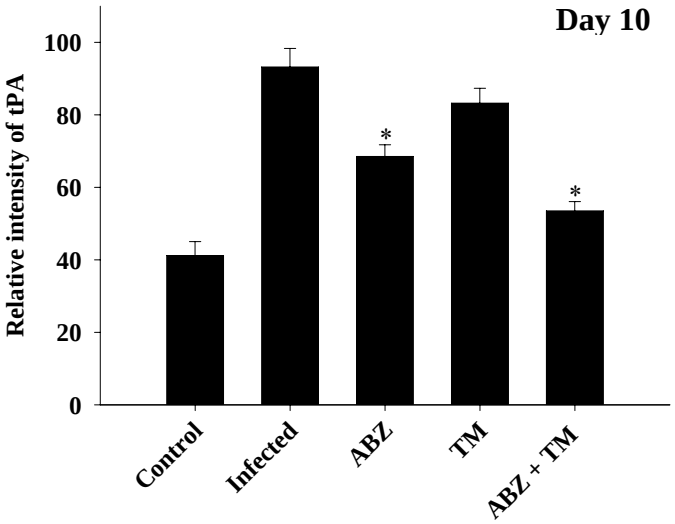
FIG. 2. Influence of treatment on eosinophil counts. (a) Treatment on day 5 PI, the CSF eosinophils were significantly increased in CSF of mice infected with *Angiostrongylus cantonensis* compared with uninfected control. Eosinophils were significantly reduced (*P<0.05) by albendazole alone or albendazole-thalidomide co-therapy, whereas mild reduction by thalidomide alone. (b) Treatment on day 10 PI, the CSF eosinophils were significantly increased in CSF of mice infected with *Angiostrongylus cantonensis* compared with uninfected control. Eosinophils were significantly reduced (*P<0.05) by albendazole alone or albendazole-thalidomide co-therapy, whereas mild reduction by thalidomide alone. (c) Treatment on day 15 PI, leukocyte were mildly reduced by albendazole alone or albendazole-thalidomide co-therapy, there was no significant difference after thalidomide treatment only. The larvae were degenated by albendazole treatment alone or albendazole-thalidomide co-therapy.

Fig. 3

(a)


Day 5
Relative intensity of tPA
100
80
60
40
20
0
*
*
Control
Infected
ABZ
TM
ABZ + TM

(b)


Day 10
Relative intensity of tPA
100
80
60
40
20
0
*
*
Control
Infected
ABZ
TM
ABZ + TM

(c)

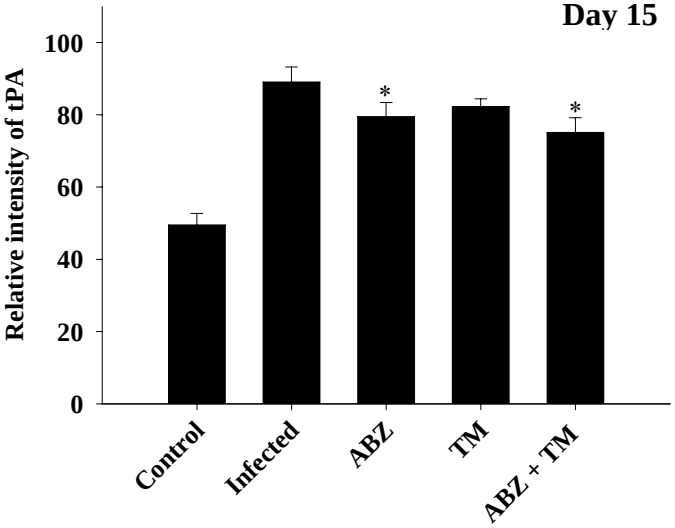

Day 15
Relative intensity of tPA
100
80
60
40
20
0
*
*
Control
Infected
ABZ
TM
ABZ + TM

FIG. 3. Influence of treatment on tPA activity. (a) Treatment on day 5 PI, the activities of tPA were significantly reduced (*P<0.05) by the individual use of albendazole (ABZ) or albendazole-thalidomide (ABZ+ TM) co-therapy, mildly reduced by thalidomide (TM). (b) Treatment on day 10 PI, the activities of tPA were significantly reduced (*P<0.05) by the individual use of albendazole (ABZ) or albendazole-thalidomide (ABZ+ TM) co-therapy, mildly reduced by thalidomide (TM). (c) Treatment on day 15 PI, the activities of tPA were mildly reduced in albendazole alone or albendazole-thalidomide co-therapy, whereas no significant changed in treatment with thalidomide alone. Quantitative analysis of the caseinolytic enzyme was performed with a computer-assisted imaging densitometer system.

Fig. 4

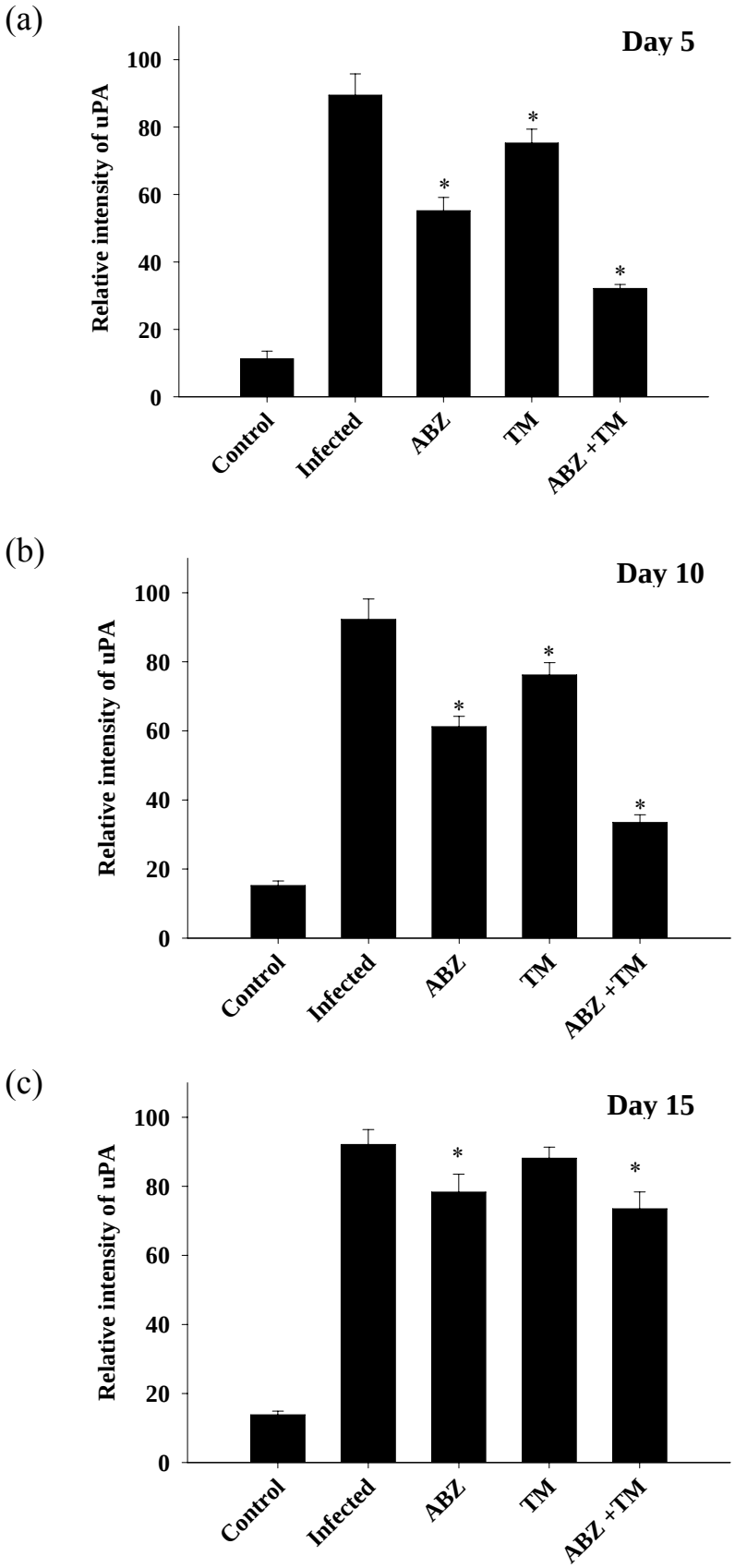

(a)
Day 5
Relative intensity of uPA
100
80
60
40
20
0
*
*
*
Control
Infected
ABZ
TM
ABZ +TM
(b)
Day 10
Relative intensity of uPA
100
80
60
40
20
0
*
*
*
Control
Infected
ABZ
TM
ABZ +TM
(c)
Day 15
Relative intensity of uPA
100
80
60
40
20
0
*
*
Control
Infected
ABZ
TM
ABZ +TM

FIG. 4. Influence of treatment on uPA activity. (a) Treatment on day 5 PI, the activities of uPA were significantly reduced (*P<0.05) by the individual use of albendazole (ABZ) or albendazole-thalidomide (ABZ+ TM) co-therapy, mildly reduced by thalidomide (TM). (b) Treatment on day 10 PI, the activities of uPA were significantly reduced (*P<0.05) by the individual use of albendazole (ABZ) or albendazole-thalidomide (ABZ+ TM) co-therapy, mildly reduced by thalidomide (TM). (c) Treatment on day 15 PI, the activities of uPA were mildly reduced in albendazole alone or albendazole-thalidomide co-therapy, whereas no significant changed in treatment with thalidomide alone. Quantitative analysis of the caseinolytic enzyme was performed with a computer-assisted imaging densitometer system.

Fig. 5

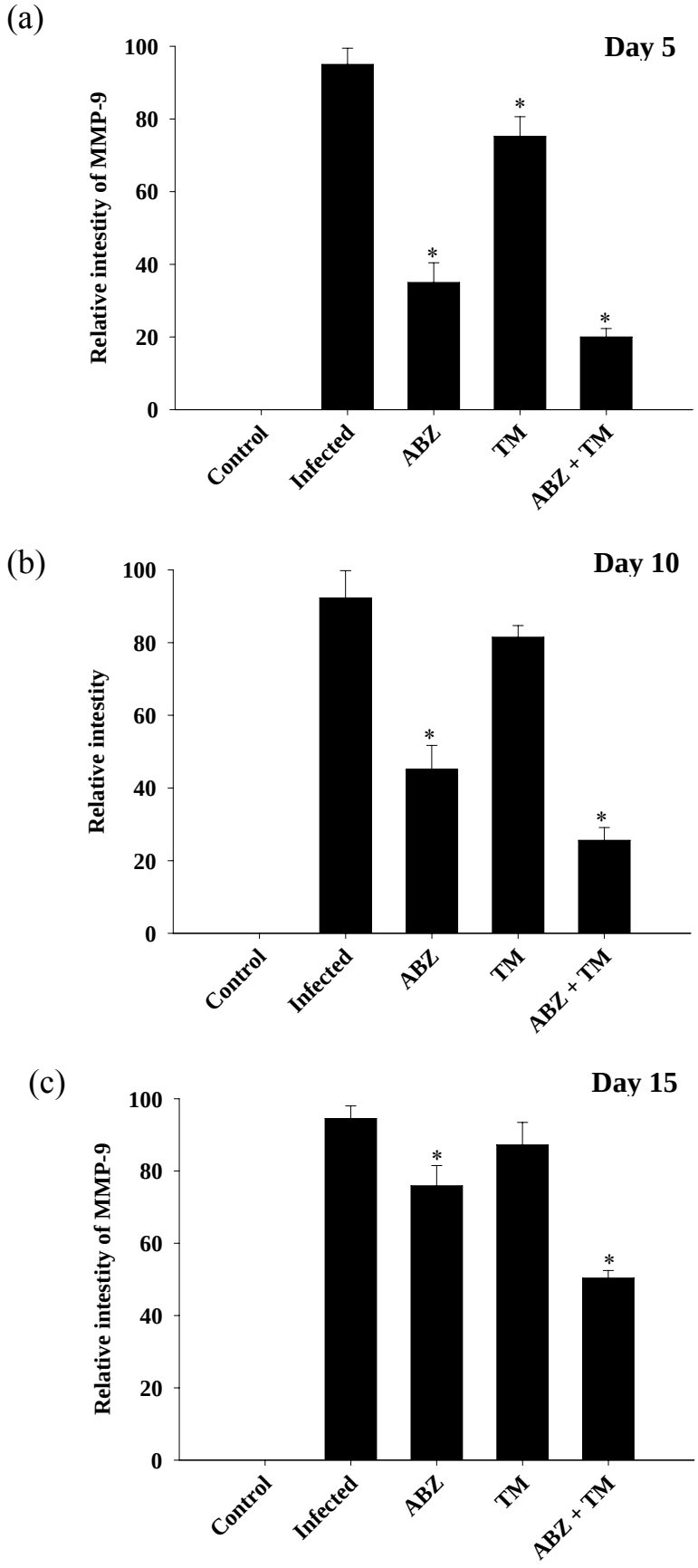

(a)
Day 5
Relative intestity of MMP-9
100
80
60
40
20
0
*
*
*
Control
Infected
ABZ
TM
ABZ + TM
(b)
Day 10
Relative intestity
100
80
60
40
20
0
*
*
Control
Infected
ABZ
TM
ABZ + TM
(c)
Day 15
Relative intestity of MMP-9
100
80
60
40
20
0
*
*
Control
Infected
ABZ
TM
ABZ + TM

FIG. 5. Influence of treatment on MMP-9 activity. (a) Treatment on day 5 PI, MMP-9 activity was significantly reduced after albendazole (ABZ) treatment alone or albendazole-thalidomide (ABZ-TM) co-therapy, and there was mild inhibition with thalidomide (TM) only. (b) Treatment on day 5 PI, MMP-9 activity was significantly reduced after albendazole (ABZ) treatment alone or albendazole-thalidomide (ABZ-TM) co-therapy, and there was mild inhibition with thalidomide (TM) only. (c) Treatment on day 15 PI, MMP-9 activity were mildly reduced in albendazole alone or albendazole-thalidomide co-therapy, whereas no significant changed in treatment with thalidomide alone. Quantitative analysis of the caseinolytic enzyme was performed with a computer-assisted imaging densitometer system.

Fig. 6

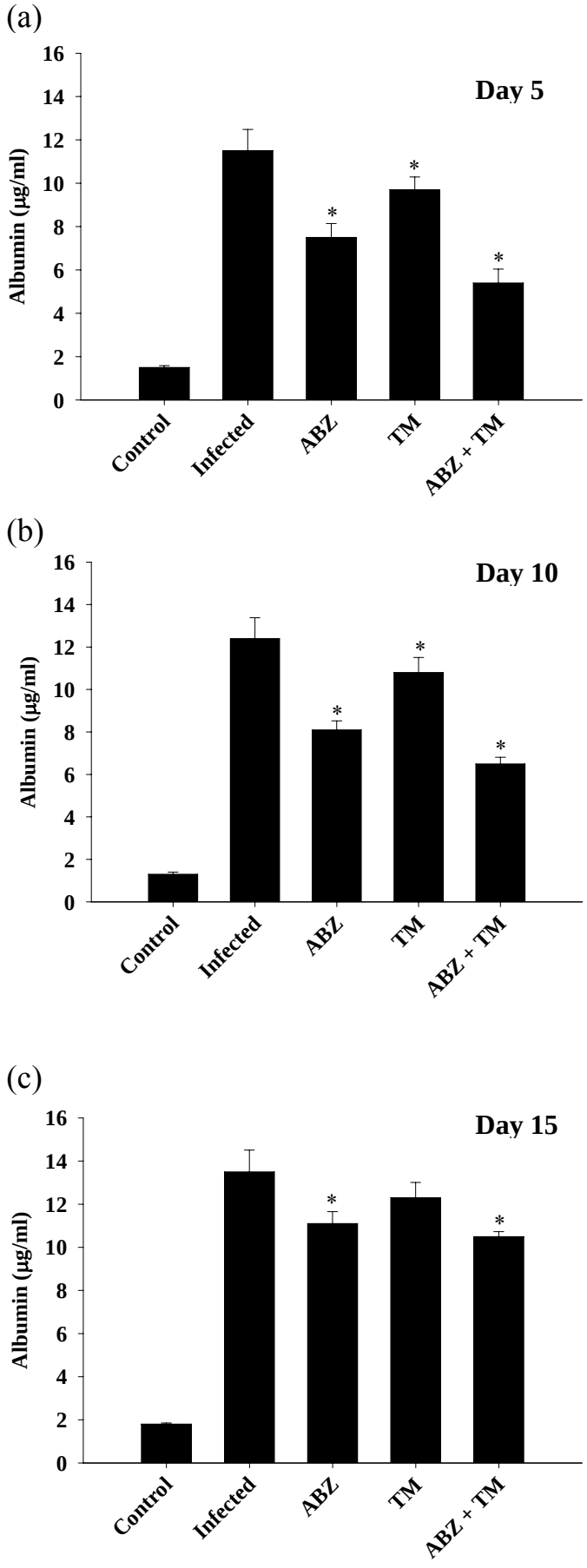

(a)
Day 5
Albumin (µg/ml)
16
14
12
10
8
6
4
2
0
*
*
*
Control
Infected
ABZ
TM
ABZ + TM
(b)
Day 10
Albumin (µg/ml)
16
14
12
10
8
6
4
2
0
*
*
*
Control
Infected
ABZ
TM
ABZ + TM
(c)
Day 15
Albumin (µg/ml)
16
14
12
10
8
6
4
2
0
*
*
Control
Infected
ABZ
TM
ABZ + TM

FIG. 6. Influence of treatment on albumin. (a) The CSF albumin was significantly increased in CSF of mice infected with *Angiostrongylus cantonensis* compared with uninfected control. Treatment on day 5 PI, the concentrations of CSF albumin were significantly less (*P<0.05) by albendazole (ABZ) alone or by albendazole-thalidomide (ABZ-TM) co-therapy mice when compared with concentrations found in infected-untreated mice, whereas mildly decreased by thalidomide (TM) treatment alone. (b) Treatment on day 10 PI, the concentrations of CSF albumin were significantly less (*P<0.05) by albendazole (ABZ) alone or by albendazole-thalidomide (ABZ-TM) co-therapy mice when compared with concentrations found in infected-untreated mice, whereas mildly decreased by thalidomide (TM) treatment alone. (c) Treatment on day 15 PI, the CSF albumin was mildly reduced by albendazole-thalidomide co-therapy, and there was no significant differences by albendazole alone or thalidomide treatment only.