• 系統編號	RC9408-0001
• 計畫中文名稱	探討抑制人類白血球誘發基質蛋白酵素活化之藥物機轉及其對活體再甦醒性傷害的保護作用之評估(I)
• 計畫英文名稱	The Study of Inhibitory Mechanisms of Anti-Leukocyte Agents on Matrix Metalloproteinase Activation and Evaluate the Protective Effects on Resuscitation Injury in Vivo
• 主管機關	行政院國家科學委員會
• 計畫編號	NSC91-2320-B038-046
• 執行機構	台北醫學院藥理科
• 本期期間	9108 ~ 9207
• 報告頁數	17 頁
• 使用語言	中文
• 研究人員	蕭哲志 Hsiao, George
• 中文關鍵字	基質金屬蛋白酵素；白血球；再甦醒性傷害
• 英文關鍵字	Matrix metalloproteinases；Leukocyte；Resuscitation injury；Andrographolide；Nuclear factor kappa-B

• 中文摘要

基質金屬蛋白酵素(Matrix metalloproteinases, MMPs)能夠催化分解維持組織結構之細胞外基質蛋白(Extracellular matrix proteins)，包括基質(Ground substances)與結締纖維組織(Connective fibers)，因此對於組織之結構重組(Remodeling)、修補(Repairing)與破壞(Destroy)都扮演相當重要之角色。同時 MMPs 的含量與活性表現均受到許多方式嚴密地調節控制。根據文獻指出，許多發炎性疾病如類風濕性關節炎的軟骨組織不正常破壞或粥狀動脈血管斑塊組織的剝離均與異常基質崩解作用有關，其主要原因源自單核球或巨噬細胞產生及釋放大量 MMPs 所致。在大規模中藥材萃取物及化學合成等藥物成分篩選實驗下，我們發現其中傳統中藥穿心蓮(Andrographis paniculata)所萃取的天然物成分 Andrographolide，具有明顯抑制 MMPs 活化之作用。在先導試驗中我們以人類單核球細胞(THP-1 cells)爲實驗細胞，分別以不同濃度的 TNF-α 或 LPS 處理 24 小時後，以電泳酵素分析法(Gelatin zymography)評估可發現到人類單核球細胞能誘發大量 MMPs 的活性，而其中又以 MMP-9 爲甚。在電泳酵素分析法下觀察到 Andrographolide 有意義地依濃度效應(1-50μM)抑制 TNF-α 或 LPS 誘發人類單核球細胞之 MMP-9 活性。然而在酵素活性分析(MMP-9 activity assay system)下，Andrographolide 在濃度 50μM 時，卻不具有抑制 MMP-9 酵素活性之能力。另外以細胞存活率測定(MTT assay)發現 Andrographolide 的抑制作用並非源自細胞之損害。並利用酵素免疫分析測定法(ELISA)，發現 Andrographolide 也會抑制 TIMP-1 的含量。從西方墨點實驗法(Western blot)發現在不同刺激下(如 TNF-α 或 LPS)發現 MMP-9 protein 表現量隨 Andrographolide 濃度增加而降低，故証實此天然物作用在 MMP-9 蛋白質表現層面。並進一步以 RT-PCR 的實驗分析法加以分析，發現 Andrographolide 會抑制 MMP-9 mRNA 的表現，更深入瞭解細胞轉錄(Transcription)之影響程度。同時我們也更進一步探討 Andrographolide 在訊息傳遞中作用機轉的方式，從實驗結果得知 Andrographolide 會明顯抑制由 TNF-α 刺激

	所導致 Inhibitor-κB-α (IκB-α)的降解作用，使 Nuclear factor-κB (NF-κB)無法進入細胞核中與特定 MMP-9 相關的 DNA 序列接合。LPS 則可能並非經 NF-κB 之途徑。在 Mitogen-activated protein kinases (MAPKs)方面，從目前實驗結果得知 Andrographolide 對於 Extracellular signal-regulated kinases (ERKs)並無直接的影響，至於 c-Jun-NH/sub 2/-terminal kinase (JNK)雖有初步結果，但仍需進一步探討其影響的程度。綜合目前實驗的結果發現，天然物 Andrographolide 的確具有抑制 MMP-9 表現之活性，而在 TNF-α 刺激方面其作用機轉可能主要藉由影響 NF-κB 的訊息傳遞過程。目前本實驗室正進行其它有效成分機轉作用評估，同時也已完成出血性再甦醒性傷害之活體動物模式。第二年將進行相關之活體實驗以瞭解這些成分(如 Andrographolide)是否具抗發炎與再甦醒性傷害之療效功能。
• 英文摘要	Matrix Metalloproteinases (MMPs) could catalyze and degrade extracellar matrix protein (ECM), including ground substances and connecting fibers, which have their function to maintain tissue structure. Thus, it lays certain important roles in tissue structure remodeling repairing and destroys. The levels and activities of MMPs are strictly regulated and controlled in various ways. Many evidence indicated that human monocytes/macrophages synthesize and secrete several MMPs which are structurally related and participate in the degradation of ECM components in either rheumatoid arthritis tissues or atherosclerotic plaques. In general, inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin 1β (IL-1β), lipopolysaccharides (LPS), can stimulate inflammatory cells to express MMPs genes and protein, and its activates are also regulated by physically endogenous tissue inhibitor of metalloproteinases (TIMPs), such as TIMP-1 and TIMP-2. According to previous experiments, we found that andrographolide extracted from Chinese herb Andrographis paniculata showed obviously inhibitory effect on MMPs activation. We used human monocyte THP-1 cells in our preliminary experiments and by using different concentration of TNF-α or LPS treatment for 24 hours. We observed that andrographolide concentration-dependently (1-50 μM) inhibit MMP-9 activation induced by TNF-α and LPS significantly in zymography method. In addition, andrographolide did not show inhibitory effect on enzymatic activity of MMP-9 at concentration of 50 μM. Also, we found that the inhibitory effect of andrographolide was not due to impairment of cellular viability by MTT tests. On the other way, andrographolide also inhibited the TIMP-1 levels by the ELISA analysis. According to Western blot method, we found that the inhibition on expression of MMP-9 protein is concentration-dependently by andrographolide in various stimulations. This indicated that this natural compound has effect on the protein expression of MMP-9. By using RT-PCR method, we found that andrographolide can inhibit the expression of MMP-9 mRNA, thus has deeper influence on the level of MMP-9 transcription. At the same time, we investigated the mechanism of action of andrographolide in various signaling pathways. We found that it could significantly inhibit the degradation of inhibitor-κB-α (IκB-α) induced by TNF-α therefore nuclear factor-κB (NF-κB) may not translocate for transcription. However, LPS is possibly not associated with this NF-κB pathway. Furthermore, in mitogen-activate protein kinases (MAPKs) aspect, andrographolide did not show direct influence on phosphorylated activation of extracellular signal-regulated kinases (ERKs), however, it showed slightly affect on c-Jun-NH/sub 2/-terminal kinase (JNK) activation. In summary, we found that Chinese herbal compound, andrographolide, with inhibitory effect on MMP-9 expression, and its main mechanism of action might through NF-κB signal pathway on TNF-α stimulation. It will be interesting to investigate its therapeutic profile on inflammatory animal model such as resuscitation injury in vivo on the second year.