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• 計畫中文名稱	探討抑制人白血球誘發基質屬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 (II)
• 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 (II)
• 主管機關	--
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• 中文關鍵字	基質屬酵素; 細胞訊息; 再甦醒性傷害
• 英文關鍵字	Matrix metalloproteinases; Signal transduction; Resuscitation injury
• 中文摘要	Matrix metalloproteinases 簡稱 MMPs，爲一群結構似且含鋅(zinc)屬子之蛋白酵素。因 MMPs 的催化作用需屬子加以活化，並且能夠催化分解維持組織結構之細胞外基質蛋白，包括基質與結締纖維組織，故稱之爲基質屬蛋白酵素，而其對於組織之結構重組、修補與破壞扮演相當重要之角色。在大規模中藥材萃取物及化學合成等藥物之成分篩選實驗中，我們發現在 histone deacetylase inhibitor II (HDI II) 及 cinnamophilin，皆 具有明顯抑制 MMPs 活化之作用。在電泳酵素分析法中觀察到 HDI II (0.01-5 M)及 cinnamophilin (1-50 M)確實有意義地依濃效應抑制對於 LPS 或 MCP-1 誘發人單核球細胞之 MMP-9 活性，此外以細胞存活測定 (MTT assay) 發現 HDI II 及 cinnamophilin 的抑制作用並非源自細胞之損害。西方點墨法 (Western blot)實驗發現在同激下(如 LPS 或 MCP-1) 細胞 MMP-9 protein 的表現會隨著 HDI II 及 cinnamophilin 濃的增加而低，故可證實此種藥物作用在 MMP-9 蛋白質表現層面。並進一步以 RT-PCR 的實驗加以分析，發現 HDI II 及 cinnamophilin 會抑制 MMP-9 mRNA 的表現，深入瞭解細胞轉 (transcription)之影響程。同時我們也進一步探討 HDI II 及 cinnamophilin 在訊息傳遞中作用機轉的方式，從實驗結果得知 cinnamophilin 會明顯抑制由 LPS 激所導致 Inhibitor- κB-α (IκB-α)的解作用，使 Nuclear factor- κB (NF-κB)無法進入細胞核中與特定 MMP-9 相關的 DNA 序接合。 在 Mitogen-activated protein kinases (MAPKs)方面，從目前實驗結果得知 HDI II 及 cinnamophilin 對於 LPS 誘發 c-Jun-NH2-terminal kinase (JNK)活化具抑制作用，但對於 extracellular signal-regulated kinases (ERKs)並無直接的 影響。 HDI II 及 cinnamophilin 對於 MCP-1 誘發細胞趨化作用具抑制效果。另外，從流式細胞儀的結果發現 HDI II 及 cinnamophilin 並會抑制 THP-1 活化後細胞表面 CD11b 的表現。活體再甦醒性傷害實驗中，的確發現實驗動物依時間關係其肝臟與腎臟功能受損。在目前活體藥物處下 HDI II 似作用藥 valproic acid 效果佳，而以抗白血球作

用藥 YC-1 具部分改善效果。綜合目前實驗的結果，發現 HDI II 及 cinnamophilin 的確具有抑制 MMP-9 表現之活性，而在 LPS 或 MCP-1 激方面其作用機轉可能主要藉由影響 NF- κB 或 JNK 的訊號傳遞過程。未也將會進 多相關之實驗及其他活體實驗以瞭解其是否具有抗發炎效之功能。

- 英文摘要

Matrix Metalloproteinases (MMPs) are a family of over 20 zinc-containing enzymes that cleave the various components of extracellular matrix. Because the catalytic ability of MMPs needs to be activated by metal ions, and because they could catalyze and degrade tissue structure maintaining extracellular matrix protein (ECM), including ground substances and connecting fibers, they are named matrix metalloproteinase. Thus, it plays an important role in tissue structure remodeling, repairing and destroys. According to previous experiments, we found that histone deacetylase inhibitor II (HDI II) and cinnamophilin showed obviously inhibitory effect on MMPs activation. We observed that HDI II and cinnamophilin significantly and concentration-dependently inhibit MMP-9 activation induced by LPS and MCP-1 by zymographic method. Also, we found that the inhibitory effect of HDI II and cinnamophilin was not due to impairment of cellular viability by MTT tests. According to Western blot method, we found that various stimulator-induced expression of MMP-9 protein is concentration-dependent inhibition by HDI II and cinnamophilin. This indicated that these two compounds have effect on the protein expression of MMP-9. By using RT-PCR method, we found that HDI II and cinnamophilin can inhibit the expression of MMP-9 mRNA, thus have deeper influence on the level of MMP-9 transcription. At the same time, we investigated the mechanism of action of HDI II and cinnamophilin in various signaling pathways. We found that cinnamophilin could significantly inhibit the degradation of inhibitor-.kappa.B-.alpha. ？\ (I.kappa.B-.alpha.) induced by LPS. Therefore, nuclear factor- ？eB (NF-.kappa.B) may not translocate for transcription. Furthermore, in mitogen-activate protein kinases (MAPKs) aspect, HDI II and cinnamophilin showed direct influence on phosphorylated activation of c-Jun-NH2-terminal kinases (JNK) activation. However, both reagents did not show direct influence on phosphorylated activation of extracellualr signal-regulated kinases (ERKs) activation. Besides, the results of flow cytometry showed that HDI II and cinnamophilin did not inhibit the expression of surface protein, CD11b, on THP-1 cells. In summary, we found that HDI II and cinnamophilin have inhibitory effect on MMP-9 expression, and its main mechanism of action might through NF-.kappa.B or MAPK signal pathway on LPS and MCP-1 stimulation. According to the resuscitation injury in vivo studies, we found the functions of liver and kidney are graduately decay by the time. Valproic acid as function as HDI II exerted slightly effect, though, anti-leukocyte agent, YC-1 exerted some protective effects. It will be interesting to further investigate its anti-inflammatory therapeutic profile via other related experiments and animal model in vivo.