探討不同藥物對基質金屬蛋白酵素活化之影響及其在人類初級與細胞株內皮細胞之差異

Investigation of the Actions of Various Compounds on Matrix Metalloproteinase Activation and the Different Effects between Primary and Cell Line Endothelial Cells

中文摘要

基質金屬蛋白酵素(matrix metalloproteinase, MMP)是一種能夠分解細胞外基質蛋白的水解酵素，包括基質與結締纖維組織，因而對於組織之結構重組、修補與破壞都扮演相當重要之角色。同時 MMP 的含量與活性表現受到許多方式嚴密地調節控制。許多文獻指出，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以及癌細胞的生長與惡性轉移皆與異常基質崩解作用有關，其主要原因源自相關細胞(血管內皮細胞、癌細胞、單核球或巨噬細胞)產生及釋放大量 MMPs 所致。一般而言，發炎性細胞激素以及生長因子等，均會刺激細胞表現 MMPs 基因及其酵素蛋白之生合成。

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 EM-1 及 VC-1 等藥物，具有抑制 MMPs 活化之作用。本實驗利用人類臍帶靜脈內皮細胞(HUVEC)的初代細胞與細胞株 ECV304 爲實驗細胞，以生長因子 bFGF 或氧化性膽固醇 7-ketocholesterol 爲刺激劑，分別以不同濃度處理 6 小時後，利用電泳酵素分析法(gelatin zymography)發現可增強 HUVEC 細胞 MMPs 的活性，特別是在 bFGF 濃度爲 20 ng/ml 及 7-ketocholesterol 濃度爲 20 mg/ml 時得到的效果是最明顯，而其中又以 MMP-2 爲主要。然而並無法增強 ECV304 MMPs 的活性。隨後以此爲試驗條件，在電泳酵素分析法下可觀察到 EM-1 與 VC-1 等藥物有意義的依濃度效應抑制內皮細胞 MMP-2 的活性。

從西方墨點法(Western blot)發現 MMP-2 蛋白質表現量隨 EM-1 與 VC-1 濃度增加而降低，故證實此二藥物作用在 MMP-2 蛋白質表現層次，且可能藉由抑制 p38 MAPK 的磷酸化來達到抑制 MMP-2 的效果。另外我們也進行體外血管新生試驗來檢測此二藥物對血管新生作用是否有抑制作用，發現 EM-1 與 VC-1 等藥物可抑制內皮細胞在 Matrigel 中形成類微血管的構造，至於其作用機制是否與抑制 MMP-2 有關，仍須進一步探討。

英文摘要

Matrix metalloproteinases (MMPs) can catalyze and degrade extracellular matrix (ECM), including ground substances and connecting fibers, which have their function to maintain tissue structure. Thus, MMPs play several important roles in tissue remodeling, repairing and destroys. The levels and activities of MMPs are strictly

regulated and controlled in various ways. Many evidences indicate that human endothelial cells, cancer cells, monocyte and macrophages synthesize and secrete several MMPs which participate in the degradation of ECM components in rheumatoid arthritis tissues or atherosclerosis or during cancer growth and metastasis. In general, inflammatory cytokines and several growth factors can stimulate MMPs gene expression and biosynthesis.

According to previous experiments, we found that EM-1 and VC-1 showed obviously inhibitory effect on MMPs activation. We use human umbilical vein endothelial cells and cell line ECV304 as experimental cell and use basic fibroblast growth factor and 7-ketocholesterol as stimulator to exam the effect on MMPs by zymography analysis. We found that bFGF- or 7-ketocholesterol-stimulated HUVEC could significantly induce MMPs activities, especially MMP-2. We found, however, no MMPs induction by ECV304 when stimulated by bFGF or 7-ketocholesterol. The gelatin degradation effect is most appropriate and significant at the condition when both bFGF and 7-ketocholesterol stimulation concentration at 20 ng/ml and 20 mg/ml. Then, based on this experimental condition, we found a dose-dependent inhibitory effect on MMP-2 activation induced by bFGF and 7-ketocholesterol.

According to Western blot analysis, we found that the inhibition on expression of MMP-2 is concentration-dependently by EM-1 and VC-1 in various stimulations. This indicated that these two compounds have effects on MMP-2 protein expression. And then we found that these two drugs exert their function by inhibit p38 phosphorylation. We also use in vitro angiogenesis assay to test if these two compounds can inhibit capillary tube formation by HUVEC on matrigel, and the result have shown that EM-1 and VC-1 can inhibit the ability of tube formation by HUVEC on matrigel. Further investigations are required to clarify the inhibitory mechanisms of these two drugs.