YC-1 抑制腫瘤壞死因子對人類單核球細胞 (THP-1) 誘發第九型基質金屬酵素活性之探討.

YC-1 inhibits TNF-a-induced the activation of matrix metalloproteinase-9 in THP-1 cellss

中文摘要

Matrix metalloproteinases (MMPs) 爲一群結構類似含鋅金屬離子之蛋白酵素,它們能夠催化分解維持組織結構之細胞外基質蛋白(extracellular matrix proteins, ECM)，如proteoglycans、collagen、elastin 及 laminin 等。因此對於組織之結構重組 (remodeling)、修補 (repairing) 與破壞 (destroy) 都具有相當重要之角色。同時 MMP 的含量與活性表現均受到許多方式嚴密地調節控制。根據許多文獻了解到類風濕性關節炎或血管斑塊不正常破壞且崩解基質的作用，主要源自單核球或巨噬細胞產生及釋放大量催化性基質金屬蛋白酵素。一般而言，發炎性細胞激素 (inflammatory cytokines)，如 Interleukin-1 (IL-1)、 platelet-derived growth factor (PDGF) 及 tumor necrosis factor-a (TNF-a) 均會刺激 MMP 的合成，但同時也會受生理性之內生性組織抑制劑如 TIMP-1 及 TIMP-2 所調節。

在大規模藥物篩選實驗下，我們發現其中 YC-1，一種目前已知之 soluble guanylylcyclase (sGC) 的活化劑，具有明顯抑制 MMP 活化之作用。我們以 THP-1 cells 爲實驗細胞，分別處理不同濃度之細胞激素 TNF-a 24 及 48 小時後，發現在刺激 24 小時後，以電泳酵素分析法 (gelatin zymography) 可發現到單核球細胞能誘發大量 MMPs 的活性。尤其 TNF-a 濃度爲 10 nM 時得到的效果最爲明顯適當，而其中又以 MMP-9 爲甚。此外，細胞數我們分別用 3 x106 cell/ml、2 x106 cell/ml 和 1 x106 cell/ml 來進行實驗得知細胞濃度以 2 x106 cell/ml 所得效果最適當。其後再以電泳酵素分析法(Zymography)觀察到 YC-1 可明顯且依濃度效應（0.5-10 mM）抑制 TNF-a 誘發人類單核球細胞（THP-1 cells）之 MMP-9 活性，其抑制 50% 反應之濃度 (IC50) 爲 1.0 ±0.4 mM，而這些抑制作用又都並非源自細胞之損害。此外亦以西方墨點法 (Western blot) 明顯觀察到 MMP-9 protein 表現量隨 YC-1 劑量增加而抑制。另外，TIMP-1 protein 則無明顯影響。爲了瞭解此抑制作用是否與 YC-1 促使 cGMP 量升高的作用有關，故加入 1H-(1,2,4)-oxadiazolo (4,3-a)-quinoxalin-1-one (ODQ， sGC 的抑制劑) 及 sodium nitroprusside (SNP， NO donor) 分別觀察 MMP-9 的活性是否受影響，由實驗得知並未改變原先的結果，故其抑制作用並非經由影響 cGMP 而來。

除此之外，在 RT-PCR 的實驗分析下，當給予 YC-1 (1 mM) 後亦會抑制 MMP-9 mRNA 的表現。同時我們也更進一步探討單核球細胞受 TNF-a 刺激下,細胞是

否可能藉由 Nuclear factor-kB (NF-kB) 或 Mitogen-activated protein kinases (MAPKs) 活化以影響 MMP-9 之產生與活化之機轉。從結果得知 YC-1 作用後會抑制 IkB-a 的降解作用，而使 NF-kB 無法進入核中與特定的 DNA 序列接合。未來更將再進一步探討 YC-1 的抑制機轉是否經由影響 MAPKs 的活化有所相

英文摘要

Matrix Metalloproteinases (MMPs) are a family of zinc-containing proteinases, and they could degrade extracellular matrix proteins (ECM), for example, proteoglycans, collagen, elastin and laminin. Thus, it is an important role for remodeling, repairing and destroy. And the levels and activities of MMPs are regulated and controlled by various ways. Many evidences show that human monocytes/ macrophages synthesize and secrete several MMPs which are structurally related and participate in the degradation of extracellular matrix components in either rheumatoid arthritis tissues or atherosclerotic plaques. In general, inflammatory cytokines, for example, Interleukin-1 (IL-1), platelet-derived growth factor (PDGF) and tumor necrosis factor-a (TNF-a), can stimulate the espression of MMPs, and its activity is also regulated by endogenous tissue inhibitor (TIMP-1).
According to the preliminary studies, we found that YC-1, as an activator of soluble guanylyl cyclase (sGC), could markedly attenuate MMP activation of human monocytes. We took THP-1 cells and stimulated by different concentrations of TNF-a for 24 and 48 hrs. It was found that exposure of THP-1 with TNF-a (10 nM) for 24 hrs, finally increased MMPs activation by the method of gelatin zymography, especially MMP-9. Besides, the cell concentration of 2 ×106 cell/ml was appropriate for our study. YC-1 could concentration-dependently (0.5-10 mM) inhibit TNF-a-induced MMP-9 activation on human monocytes (THP-1 cells) with an IC50 value of 1.0 ±0.4 mM. The inhibitory activiyies of YC-1 were not mediated by reduction of cellular viability. In addition, we found that YC-1 could concentration-dependently inhibit expression of MMP-9 protein, and without any significant effect on the expression of TIMP-1 protein. In order to understand whether the inhibitory activity of YC-1 through increasing cGMP levels, either 1H-(1,2,4)-oxadiazolo (4,3-a)-quinoxalin-1-one (ODQ, inhibitor of sGC) or sodium nitroprusside (SNP, NO donor) was tested. It was interesting that inhibitory effect of YC-1 was not abrogated by ODQ. Additionally, induction of gelatinolytic action by TNF-a was not attenuated by SNP.
Furthermore, we investigate if YC-1 affected the expression of messenger RNA (mRNA). The expression of MMP-9 mRNA was inhibited by YC-1 (1 mM) at a reduction of 70 % by the reverse transcription-polymerase chain reaction (RT-PCR).

Also, we investigated the inhibitory mechanism of YC-1 on the signal transduction of TNF-a. We found that YC-1 could inhibit the degradation of IkB-a. Together, our findings revealed that YC-1 decreases MMP-9 expression in human monocytic cells through inhibition of nuclear factor kappa-B (NF-kB) activation, which may occur independent of cGMP.