探討比較 YC-1 等藥物對 TGF-beta1 刺激 THP-1 誘發 MMP-2 之抑制機轉

Investigation of the Inhibitory Mechanisms of YC-1 and Other Related Drugs on Matrix Metalloproteinase-2 Induced by TGF-beta1 in THP-1 Cells

中文摘要

許多證據顯示人類單核球細胞(THP-1)和巨噬細胞可以分泌合成出結構類似且含有鋅(Zinc)金屬離子之內生性蛋白水解酵素。這群水解酵素稱之爲基質金屬蛋白酵素(matrix metalloproteinases，MMPs)，他們具有可以分解及破壞細胞外基質(extracellular matrix，ECM)之能力，這會導致大部分的 ECM 沉積。而過多的 ECM 沉積，通常是發炎性疾病和纖維性病變的一種顯著特徵。特別的是，當 MMPs 和 TIMPs (一種會和 MMPs 互相制衡的蛋白質)之間失去平衡機制，這是被認爲造成基質沉積，繼而導致許多不同的器官組織纖維化，例如：肺臟、肝臟、心臟和腎臟所最主要的因素。

因此 MMPs 對於組織的重組(remodeling)、修補(repairing)與破壞(destroy)上扮演著相當重要之角色。而最近研究證據顯示出在一些神經退化性疾病的病理過程中，例如：阿滋海默症(Alzheimer's disease)等，皆與 MMPs 有關。另一方面，在癌症中，MMPs 也參與著癌細胞生長等一連串的許多過程，且能使癌細胞在轉移(metastasis)入侵的進程中滲透周圍組織。

我們發現由人工合成的 YC-1 (一種 nitric oxide-independent 和 superoxide-sensitive 的 soluble guanylyl cyclase 活化劑)具有明顯抑制 MMPs 活性的能力。在實驗中利用電泳酵素分析法 (zymography method)以及西方墨點法 (Western blot)發現，在人類單核球細胞(THP-1)中 YC-1 可以抑制轉型成長因子(Transforming growth factor-beta1，TGF-beta1)所引起的 MMP-2 酵素活性及表現。在電泳酵素分析法(gelatin zymography)下，我們觀察到由 TGF-beta 刺激 MMP-2 酵素之活化會隨著 YC-1 濃度增加皆可有效地被抑制。接著利用細胞存活率測定(trypan blue exclusion method)可發現 YC-1 的抑制作用並非源自細胞之損壞或死亡。而後以西方點墨法(Western blot)之實驗方法，發現 YC-1 濃度增加皆可有效地抑制由 TGF-beta1 刺激 MMP-2 蛋白之表現量。此外在 enzyme-linked immunosorbent assay (ELISA)實驗中發現，YC-1 於較高濃度 0.5 microM 時能有意義地降低 TIMP-1 之產生。而在 TIMP-2，甚至只要 0.1 microM 時就能有意義地降低 TIMP-2 之產生。在轉錄 (transcription)層級方面，利用反轉錄－聚合酵素連鎖反應(Reverse transcription-polymerase chain reaction)，YC-1 可以隨著濃度效應壓制 TGF-beta1 所引起的 MMP-2 mRNA 的表現。

而我們另外以西方點墨法(Western blot)之實驗方法，發現 YC-1 濃度增加，並無法抑制由 TGF-beta1 刺激而產生磷酸化的 Smad2 和抑制 Smad4 的轉位，需要更進一步的研究。

綜合目前之實驗結果，發現 YC-1 確實能選擇性地抑制人類單核球細胞(THP-1 cells)中，TGF-beta1 所誘發的 MMP-2 活性與表現，而此抑制之機轉可能主要是影響 Smad 之訊息傳遞

路徑。然而對於 YC-1 抑制人類單核球細胞的增生機轉尚需更深入地探討。因此將來對於此成分在活體中，是否能在抗發炎作用有所助益是有趣並值得去深入研究的。

英文摘要

Many evidences indicate that human monocytes (THP-1) and macrophages synthesize and secrete a family of zinc-dependent endopeptidases named matrix metalloproteinases (MMPs) and they can degrade and disrupt most components of the extracellular matrix (ECM), consequently resulting in most extracellular matrix deposition. Excessive accumulation of the extracellular matrix is a hallmark of many inflammatory and fibrotic diseases. In particular, an imbalance between MMPs and Tissue inhibitor of matrix metalloproteinases (TIMPs) is thought to be a main cause of increased matrix deposition, consequently resulting in tissue fibrosis within different organs, including lung, liver, heart, and kidney.

Therefore MMPs play an important role in matrix remodeling, repairing and destroying. Recent evidence has indicated that MMPs are involved in the pathogenesis of neurodegenerative diseases as Alzheimer's disease. On the other hand, in cancer, MMPs encourage tumor cells to penetrate the surrounding tissue during the invasive process of metastasis and can mediate many processes in tumor growth and progression.

We found that YC-1 (a nitric oxide-independent and superoxide-sensitive activator of soluble guanylyl cyclase) from artificial medicines showed obviously inhibitory effect on MMPs activation. In this study, we found that YC-1 was shown to inhibit the Transforming growth factor-beta1(TGF-beta1)-induced MMP-2 activation and expression in human monocyte THP-1 cells by zymography method and Western Blot. According to gelatin zymography method, we found that the activation of MMP-2 protein induced by TGF-beta1 were inhibited by YC-1 in a concentration-dependent way. We also found that the inhibitory effect of YC-1 was not due to impairment of cellular viability measured by trypan blue exclusion method. According to Western blot analysis, we observed that the inhibition on TGF-beta1-induced expression of MMP-2 protein by YC-1 is concentration-dependent. Furthermore, we found at 0.5 microM, YC-1 could significantly reduced TIMP-1 proteins measured by enzyme-linked immunosorbent assay (ELISA). On the other hand, we also found even at 0.1 microM, YC-1 could significantly reduced TIMP-2 proteins measured by enzyme-linked immunosorbent assay (ELISA). In the transcription level, the result that YC-1 suppressed the TGF-beta1-induced MMP-2 mRNA expression is concentration-dependent inhibition by using RT-PCR.

According to Western blot analysis, we observed that the inhibition both on

TGF-beta1-induced phosphorylation of Smad2 protein and translocation of Smad4 protein by YC-1 are concentration-independent. It needs further study.

In summary, we found that YC-1 had inhibitory effect on MMP-2 expression and activation in THP-1 cells. Its main mechanism of action might be through Smad signal pathway on TGF-beta1 stimulation. However, the inhibitory mechanisms of YC-1 on THP-1 need further investigated. It will be interesting to study further the anti-inflammatory activities of this compound on inflammation-related disease in vivo.