探討馬兜鈴酸對第九型基質金屬蛋白酵素活化與非特異性免疫功能作用的機轉

INVESTIGATION OF THE EFFECTS OF ARISTOLOCHIC ACID ON MONOCYTIC MATRIX METALLOPROTEINASE-9 ACTIVATION AND NON-SPECIFIC IMMUNE FUNCTION

中文摘要

許多研究顯示，類風濕性關節炎是一個包含免疫及發炎系統的疾病，而其最大的特徵便是骨骼及軟骨的崩解破壞。而在類風濕性關節炎病患的滑液膜中可發現單核球/巨噬細胞、淋巴球及纖維母細胞的數量大量增加，白血球及滑液之纖維母細胞會過量分泌許多前發炎細胞激素(pro-inflammatory cytokines)，因而導致慢性發炎。這些細胞素中主要包括腫瘤壞死因子(tumor necrosis factor-alpha, TNF-)、interleukin (IL)-1 及 IL-6。它們可以活化各式各樣的基因表現，包括合成不同細胞激素，以及和組織降解有關的基質金屬蛋白酵素 (matrix metalloproteinases, MMPs)。而 MMPs 在腫瘤轉移中也扮演著重要的角色。腫瘤細胞的轉移主要是因爲其可以分解途中細胞外基質 (extra cellular matrix, ECM)中主要的組成蛋白；MMPs 也參與了癌細胞早期的發展，其中包含刺激細胞增生及血管增生的調節等。

我們發現由中草藥 (如 Aristolochia spp.)所萃取出的馬兜鈴酸(aristolochic acids, AsA)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)中馬兜鈴酸可以抑制腫瘤壞死因子(TNF-) (10 ng/ml)所引起的 MMP-9 酵素活性及表現(IC50 爲 6.41 ± 0.45 M)。而馬兜鈴酸主要具有兩種不同的組成物－aristolochic acids I 及 II。由於已知馬兜鈴酸具有 phospholipase A2 抑制作用，所以我們也利用了 indomethacin (10、20 M)來排除馬兜鈴酸的 MMP-9 抑制作用是否經由前列腺素(prostaglandin)路徑的可能。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)，馬兜鈴酸可以壓制 TNF- 所引起的 MMP-9 mRNA 的表現。更進一步地探討，馬兜鈴酸也抑制了 TNF- 所引起的 I B- 的降解作用。在細胞核內方面，利用電泳移動偏向分析法 (electrophoretic mobility shift assay, EMSA)發現，馬兜鈴酸可以抑制 TNF- 所引起的 NF- B 活化現象，而在 NF- B 轉位(translocation)中也有部分抑制情形。另一有趣的現象是，馬兜鈴酸在小鼠脾臟細胞中可以抑制 concanavalin A (Con A)及 lipopolysaccharide (LPS)所引發的細胞增殖行爲。我們也將此抑制效果與一已知固醇類藥物－dehydroepiandrosterone (DHEA)的抑制效果做比較，發現其抑制效果相當。此外淋巴細胞經由 Con A 所引發之細胞激素 (IL-2 及 interferon-)的釋出也可被馬兜鈴酸依濃度所影響，然而對於馬兜鈴酸抑制淋巴細胞的增生機轉尚需更深入地探討。因此將來對於此天然成分在活體中類風濕性關節炎所引起之滑液膜傷害，是否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 evidence indicated that rheumatoid arthritis (RA) is a disease including immune and inflammatory systems which are linked to the destruction of cartilage and bone. Monocyte/macrophages, lymphocytes and fibroblasts are found in highly increased numbers in the synovial membrane in RA. Leukocytes and synovial fibroblast overproduce pro-inflammatory cytokines, mainly tumor necrosis factor-alpha (TNF-), interleukin (IL)-1 and IL-6, which lead to chronic inflammation. These cytokines activate a variety of gene expression, including genes coding for various cytokines and matrix metalloproteinases (MMPs) involved in tissue degradation. On the other way, MMPs also play an important role in tumor invasion. The invasive properties of tumor cells, owing to their ability to degrade all major protein components of the extracellular matrix (ECM) and basement membranes. They also participate in early steps of tumor evolution, including stimulation of cell proliferation and modulation of angiogenesis.

We found that aristolochic acids (AsA) extracted from herbal medicines (such as Aristolochia spp) showed obviously inhibitory effect on MMPs activation. In this study, we found that AsA was shown to inhibit the TNF- -induced MMP-9 activation and expression in human monocyte THP-1 cells by zymography method and Western Blot (IC50= 6.41 ± 0.45 M). We also found the different ratio (I : II = 55:42 and 29:66) of aristolochic acids I and II with similar effect on inhibition of MMP-9 activation. We also used indomethacin to exclude the inhibitory action of AsA involved in prostaglandin pathway. In the transcription level, AsA suppressed the TNF- -induced MMP-9 mRNA expression by using RT-PCR. AsA also inhibit the TNF- -induced I B- degradation. In the nuclear aspect, we also found that AsA inhibited TNF- -induced NF- B activation (by EMSA method) and translocation. Interestingly, AsA was shown to concentration-dependently inhibit the concanavalin A (Con A) and lipopolysaccharide (LPS) induced proliferation of mouse splenocytes. We also compare the inhibitory effect of AsA with dehydroepiandrosterone (DHEA), and the inhibitory effect is similar. By the way, the cytokines (IL-2 and interferon-) released from the ConA-induced lymphocyte were concentration-dependently affected. However, the inhibitory mechanisms of AsA on lymphocyte need further investigated. It will be interesting to study further the anti-inflammatory activities of this nature compound on RA-related synovial injuries in vivo.