Matrix metalloproteinase-9 抑制血小板凝集作用之機轉探討

Mechanisms involved in the antiplatelet activity of matrix metalloproteinase-9

中文摘要

MMP-9 (matrix metalloproteinase-9) 會影響血管新生與心血管疾病的發生。通常人體在動脈硬化破裂 (atherosclerotic plaque rupture) 、動脈瘤形成、動脈硬化症 (atherosclerosis) 、再窄化 (restenosis) ，心肌肥大 (dilated cardiomyopathy) 及心肌梗塞 (myocardial infarction) 時都會產生大量的 MMP-9。然而，在心血管系統中佔重要地位的血小板，在受到刺激活化的過程中是否存在著 MMP-9 的影響，至今仍未有一套完整的研究可加以證實。本研究係探討 MMP-9 在血小板活化過程中對細胞內一些訊息傳遞的影響，亦即抑制血小板凝集的機轉。經此一系列實驗後，研究結果顯示：(1) 在 in vitro 實驗中已發現外加 activated MMP-9 確實具有抑制血小板凝集之能力。亦即在血小板凝集實驗中若外加 activated MMP-9，則會隨著濃度之增加而有效地抑制由 collagen、ADP、arachidonic acid、thrombin 與 U46619 等血小板活化劑所引起的凝集作用，隨著血小板活化劑使用種類的不同， MMP-9 之 IC50 約爲 21~60 ng/ml。(2)同時 MMP-9 可有效的抑制由 collagen 刺激之血小板活化所引起的細胞內鈣離子移動與 phosphoinositide breakdown。(3) MMP-9 可促進 cAMP、NO 與 cGMP 之增加，抑制 TxB2 之形成，降低細胞膜流動性與細胞內 pH 值。(4)對於血小板中之 47 kDa 蛋白質磷酸化，這是一個標記 protein kinase C 活性的方法，在本實驗中我們分別使用 collagen (2 g/ml) 和 PDBu (0.06 M) 促進血小板 47 kDa 的蛋白質磷酸化；由研究結果顯示 MMP-9 可抑制其活性。

由結果發現 MMP-9 抗血小板活性可能涉及以下路徑：(1) MMP-9 在一開始會改變血小板細胞膜之流動性，而抑制 phospholipase C 的活性，接著進一步抑制 phosphinositide breakdown 、 47kDa 的 PKC 之磷酸化和 thromboxane A2 (TxA2)的形成； (2) 再者 TxA2 含量的減少會增加血小板細胞膜上 adenylate cyclase 的活性，而增加 cyclic AMP 的量， cAMP 的增加會進一步抑制 PLC 的活性,使得 PKC 對 47 kDa protein 的 phosphorylation 受到影響，同時亦會抑制細胞內鈣離子的移動； 此外，cyclic AMP 量增加的同時亦可抑制由 thrombin 和 ADP 所刺激之 Na+/H+ 交換,導致細胞內 pH 值的降低，進一步抑制細胞內鈣離子的增加。(3) 另一方面，MMP-9 可能經由活化血小板內 NO synthetase 產生 NO，活化 guanylate cyclase，使 cGMP 的含量增加。藉由上述 (1)、(2) 和 (3) 的作用最後導致細胞內鈣離子之濃度的減少，最後抑制血小板凝集反應。

英文摘要

MMP-9 plays an important role in angiogenesis and cardiovascular diseases. There are a lot amount of MMP-9 detected in atherosclerotic plaque rupture、atherosclerosis、restenosis、dilated cardiomyopathy and myocardial infarction. However, the exact mechanism is still unclear and requires further characterization. In this study, we found that (1) the activated MMP-9 can effectively inhibit the platelet aggregation. MMP-9 dose-dependently inhibited the aggregation induced by collagen, ADP, AA, thrombin and U46619, the IC50 concentration are about 21-60 ng/ml. (2) MMP-9 significantly inhibited intracellular Ca2+ mobilization and PI breakdown stimulated by collagen. (3) MMP-9 increased the cAMP, cGMP, and NO formation in human platelets. In addition, MMP-9 also decreased the formation of TxB2, membrane fluidity and the intracellular pH values. (4) MMP-9 also significantly inhibited platelet aggregation and decreased the 47 kDa protein phosphorylation induced by PDBu, an PKC activator.

Therefore, base on the above observations, we suggest that the possible mechanisms maybe involved as follows : (1) MMP-9 altered membrane fluidity is the primary mechanism, followed by the inhibition of phospholipase C, and then inhibited the phosphoinositide breakdown, 47 kDa phosphorylation and formation of TxA2.(2) Furthermore, the decrease of TxA2 will increase the adenylate cyclase activity, thereby leading to the inhibition of phospholipase C .(4) The increase of cAMP inhibited the exchange of Na+/H+ stimulated by thrombin, and then decreased the intracellular pH value.(5) On the other hand, MMP-9 increased the amount of NO and c-GMP by in this study.