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• 計畫英文名稱	Comparison of the Relative Effect of Triflavin with Various Anti- Integrin Monoclonal Antibodies in Fibrin Clot Retraction
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• 英文關鍵字	Snake venom protein；Integrin；Fibrin clot；Monoclonal antibody；Endothelial cell；Retraction
• 中文摘要	血漿中纖維蛋白凝塊的回縮作用(Fibrin clot retraction),對分解血塊而言,扮演著一個非常重要的角色。另外,此回縮作用亦可幫助受傷的血管儘快恢復通暢,以保持血流的穩定。對生理上的意義而言,Fibrin clot retraction 可加強血塊的分解和促進血管再 Recanalization。在作用機轉上,雖然詳細的機轉仍然不是很清楚,但由富含血小板的血漿中清楚可知,是由靜態且非移動性的纖維蛋白網和動態且具主動移動性質的血小板,兩者間相互作用造成的。最近,更進一步證實血小板細胞膜上的 Fibrinogen receptor,即.alpha./sub IIb/.beta./sub 3/ integrin(醣蛋白 IIb/IIIa complex)參與了此血小板-依賴型的凝塊回縮作用(Platelet-dependent fibrin clot retraction)。因此,抗.alpha./sub IIb/.beta./sub 3/ integrin 的單源抗體及可專一性結合到.alpha./sub IIb/.beta./sub 3/ integrin 上的合成 Peptide,可作爲一理想的研究工具來研究在血塊回縮反應中,血小板.alpha./sub IIb/.beta./sub 3/ integrin 在其中所扮演的角色。由目前已知的研究結果得知,血小板活化後可幫助 Fibrin clot retraction,其中血小板可能經由其表面上的.alpha./sub IIb/.beta./sub 3/ integrin 直接和 Fibrin 結合;另一方面,則可能藉由如血小板細胞內的蛋白質骨架重組作用(Cytoskeletal assembly)而造成此回縮作用。 .alpha./sub IIb/.beta./sub 3/本身爲.beta./sub 3/ integrin 的一員,屬於一種附著蛋白受體;它在體內參與了發育(Development)、發炎(Inflammation)、腫瘤細胞轉移(Tumor cell metastasis)及血栓(Thrombosis)等等的反應。.alpha./sub v/.beta./sub 3/也是.beta./sub 3/ integrin 的一員,廣泛的存在於如內皮細胞(Endothelial cell)和纖維母細胞(Fibroblast)上。因爲.alpha./sub v/.beta./sub 3/和.alpha./sub IIb/.beta./sub 3/ integrin 一樣可和 Fibrinogen 結合,同時亦含有此.beta./sub 3/ subunit;因此,在 Fibrin clot retraction 中, .alpha./sub v/.beta./sub 3/ integrin 可能亦扮演某種角色,值得進一步研究證實。 Triflavin 爲一種由出血性的蛇毒 Trimeresurus flavoviridis 中所分離出的強效抗血小板凝集蛋白;它本身爲單鍵含有 70 個 Amino acid;在靠近 C 端位置,也就是在 49-51 的位置上含有 Arg-Gly-Asp 這三個 Amino acid,在 Triflavin 抑制血小板凝集過程中扮演了決定性的角色。Triflavin 的作用機轉爲競爭性的抑制 Fibrinogen 和血小板.alpha./sub IIb/.beta./sub

3/ integrin 的結合作用;爲一種專一性的.alpha./sub IIb/.beta./sub 3/ integrin 拮抗劑。它能強力的抗血小板凝集,因此在活體內亦能有效的防止血栓的產生。由本研究發現,血小板(1*10/sup 9//ml)及人類臍靜脈內皮細胞(HUVEC,1*10/sup 7/ /ml)可明顯的促進纖維蛋白絲的回縮;且兩者的作用活性差不多。另外,我們亦發現 Triflavin(1.mu.M)可明顯的抑制 80%血小板引起的 Fibrin clot retraction,而 anti-P-selectin 單源抗體則無明顯抑制活性。進一步研究發現,Triflavin(1.mu.M)亦可抑制內皮細胞所引起的回縮反應,且其抑制程度約與 anti-.alpha./sub v/.beta./sub 3/單源抗體相似。 經由上述的研究可使我們更清楚的瞭解血小板及內皮細胞在 Fibrin clot retraction 過程中的角色,特別是血管內皮細胞上的 Integrin .alpha./sub v/.beta./sub 3/可能扮演一重要角色,而血小板上的 p-selectin 則不參與此回縮反應。

- 英文摘要

Fibrin clot retraction may be important in the resolution of thrombi. Contraction of fibrin clots helps to maintain the patency of injury blood vessels. Physiologically, clot retraction may enhance clot lysis and facilitate recanalization of blood vessels. However, the mechanism of clot retraction remains unclear, though in platelet-rich plasma it has been clearly shown to result from the interaction of a static, non-motile fibrin mesh and dynamic, actively motile blood platelets. Recently, it has been demonstrated that integrin .alpha./sub IIb/.beta./sub 3/ (glycoprotein IIb/IIIa complex; GP IIb/IIIa complex), a platelet surface receptor for fibrinogen, is essential for platelet-dependent clot retraction. The effects of anti-.alpha./sub IIb/.beta./sub 3/ monoclonal antibodies (mAbs) and synthetic .alpha./sub IIb/.beta./sub 3/ligand-mimetic peptides provided insights into the functional requirements of .alpha./sub IIb/.beta./sub 3/ for clot retraction. Platelet stimulation could support clot retraction through a direct action on .alpha./sub IIb/.beta./sub 3/ or by stimulation of other processes such as cytoskeletal assembly. .alpha./sub IIb/.beta./sub 3/ belongs to the .beta./sub 3/subfamily of integrins, adhesion receptors involved in development, inflammation, metastasis, and thrombosis. .alpha./sub v/.beta./sub 3/ is widely distributed in tissues such as endothelial cells and fibroblasts. .alpha./sub IIb/.beta./sub 3/ is relatively platelet specific, yet cells such as fibroblasts can also retract clots. .alpha./sub v/.beta./sub 3/ has fibrinogen-binding activity similar to .alpha./sub IIb/.beta./sub 3/. These considerations prompted us to examine the role of .alpha./sub v/.beta./sub 3/ in fibrin clot retraction. Triflavin, a potent platelet aggregation inhibitor, was purified from the venom of Trimeresurus flavoviridis. Its sequence is rich in cysteine and contains the Arg-Gly-Asp sequence at residues 49-51 in the carboxy terminal domain. The Arg-Gly-Asp sequence of triflavin plays an important role in mediating the binding of triflavin towards GP IIb/IIIa complex. Triflavin inhibits platelet aggregation by interfering with the interaction of fibrinogen with the GP IIb/IIIa complex (.alpha./sub IIb/.beta./sub 3/ integrin). It is an effective agent in the prevention of thromboembolism. The present study was designed to determine the effect of triflavin on fibrin clot retraction, and to compare the relative activities of synthetic GRGDS peptide, anti-P-selectin, anti-.alpha./sub IIb/.beta./sub 3/ and anti-.alpha./sub v/.beta./sub 3/ mAbs in this reaction. In this study, we found that human platelets (1*10/sup 9//ml) and HUVECs (1*10/sup 7//ml) markedly promoted the fibrin clot retraction. On the other hand, triflavin (1.mu.M) significantly inhibited platelet-induced fibrin clot retraction about 80%, whereas P-selectin monoclonal antibody did not affect this reaction. Furthermore, triflavin (1.mu.M) also significantly inhibited HUVEC-induced fibrin clot retraction, the potency is very similar to anti-.alpha./sub v/.beta./sub 3/ mAb in HUVEC-induced fibrin clot retraction.