C-Phycocyanin 抑制血小板凝集作用之機轉探討

Mechanisms Involved in the Antiplatelet Activity of C-Phycocyanin

中文摘要

藻藍素(C-phycocyanin, C-PC)是一種藻膽色素蛋白質(phyco-biliprotein)，爲藍綠藻類中螺旋藻(Spirulina platensis)的主要成分。此一蛋白質色素已被證實具有抗癌、自由基清除、抗氧化及抗發炎的特性。然而 C-PC 在血小板上的藥理學功效尚未明確，因此我們有意探討 C-PC 在血小板活化過程中，對於訊息傳遞方面的抑制作用。研究結果顯示，C-PC 隨著濃度的增加(0.5 - 10 nM)，能有效地抑制 collagen (1 g/ml)與 U46619 (1 M)所引起的人類血小板凝集反應以及 ATP 釋放反應；C-PC (4 和 8 nM)亦可顯著地抑制 collagen (1 g/ml)所引起的細胞內鈣離子移動以及 thromboxane A2 (TxA2)的生合成。另外，C-PC (4 和 8 nM)可有意義的增加人類血小板細胞內 nitrite 與 cyclic GMP 的含量，但對於 cyclic AMP 的含量並沒有影響；另一方面，C-PC (4 和 8 nM)可有效地清除由 collagen (1 g/ml)刺激所產生的 hydroxyl radicals 以及誘發 vasodilator-stimulated phosphopretein (VASP)之磷酸化。PDBu (150 nM)可誘發 protein kinase C 的活化，並且將 47 kDa proteins 磷酸化，C-PC (4 和 8 nM)可有效地抑制已標記[-32P] ATP 的人類血小板發生此磷酸化反應。

由結果證實，C-PC 抑制血小板活性的作用可能牽涉下列路徑：(一) C-PC 會刺激 NO 的生成，接著增加血小板細胞內 cyclic GMP 的含量，並且誘發 VASP 磷酸化、抑制 protein kinase C 的活性以及 47 kDa proteins 磷酸化反應。(二) C-PC 利用其清除 hydroxyl radicals 的作用，影響 phospholipase A2-cyclooxygenase 路徑的反應，進一步抑制 TxA2 的生合成。綜合以上結果，導致 C-PC 抑制血小板細胞內鈣離子的移動以及濃度的增加，最後因而抑制血小板的凝集反應。此項作用意味著 C-PC 可有效地應用在治療與血小板過度活化相關之疾病。

英文摘要

C-phycocyanin (C-PC), a phycobiliprotein, is one of the major constituents of blue-green algae, Spirulina platensis. This protein pigment has been reported to have anti-cancer, free radical scavenging, anti-oxidant and anti-inflammatory properties. However, the pharmacological functions of C-PC on platelets were not yet understood, we are interesting in investigating the inhibitory effects of C-PC on cellular signal transduction during the process of platelet activation. In this study, C-PC concentration-dependently (0.5-10 nM) inhibited collagen (1 g/ml)- and U46619

(1 M)-induced human platelets aggregation and ATP-release reaction. In addition, C-PC (4 and 8 nM) markedly inhibited intracellular Ca2+ mobilization in Fura-2 AM-loaded platelets and thromboxane A2 formation stimulated by collagen (1 g/ml). Furthermore, C-PC (4 and 8 nM) significantly increased the formations of nitrate and cyclic GMP but not cyclic AMP in human platelets. Moreover, C-PC (4 and 8 nM) obviously scavenged collagen (1 g/ml)-induced hydroxyl radicals and induced the phosphorylation of vasodilator- stimulated phosphoprotein (VASP). Rapid phosphorylation of a protein of Mr 47,000 (P47), a marker of protein kinase C activation, was triggered by PDBu (150 nM). This phosphorylation was inhibited by C-PC (4 and 8 nM) in [-32P] ATP-labeled human platelets.

In conclusion, our study suggested that the possible pathways of anti-platelet activity of C-PC may involve the following pathway: (1) C-PC stimulated nitrate formation, followed by increasing the amount of cyclic GMP and then induced VASP phosphorylation, inhibited protein kinase C activation and 47 kDa protein phosphorylation. (2) C-PC significantly inhibited thromboxane A2 formation through scavenging the hydroxyl radicals to inhibit phospholipase A2-cyclooxygenase pathway, and intracellular Ca2+ mobilization. Taken together, C-PC may be used as an effective tool in treating pathological disorder associated with platelet hyperaggregability.