Caffeic acid phenethyl ester 抑制血小板凝集作用之機轉探討

Mechanisms Involved in the Antiplatelet Activity of Caffeic acid phenethyl ester

中文摘要

Caffeic acid phenethyl ester (CAPE)是由蜜蜂收集眾多植物所得的樹酯產物，爲蜂膠的主要成分之一。此一物質已被證實具有抗癌、抗發炎、免疫調節的特性。然而在血小板上的藥理學功效尚未明確，因此我們有意探討 CAPE 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，CAPE 隨著濃度的增加 (6-25 M)，能有效地抑制 collagen (1 g/ml)所引起的人類血小板、人類富含血小板血漿凝集反應以及 ATP 釋放反應，但不影響由 thrombin (0.01 U/ml)、arachidonic acid (60 M)、U46619 (1 M)、ADP (20 M)和 epinephrine (10 M)所引起的反應。Collagen 所引起血小板凝集反應的劑量反應曲線可以因爲 CAPE (15-100 M)的加入而有右移的現象，由 Schild plot 分析可得 pA2 爲 4.28，斜率爲 0.826。Convulxin 是從 C. durissus terrificus 蛇毒中所純化出來的，是 collagen 受體 GPVI 的致效劑；而強力血小板活化物質 aggretin 是從 Calloselasma rhodostoma 蛇毒中所純化，可以活化 integrin 2 1。CAPE 也可以抑制 convulxin (100 ng/ml)和 aggretin (3.6 g/ml)所引起的血小板凝集反應。FITC-collagen 的螢光量可以因爲 CAPE (25 M)的加入而減少，意味著 CAPE 可以可以和 collagen 競爭受體。在 CAPE 的存在下，血小板吸附到 collagen 上會成濃度相關的減少。CAPE (15 和 25 M)可以抑制由 collagen 所刺激細胞內鈣離子的流動、phosphoinositide 的增加和 thromboxane A2 的形成，除此之外，CAPE (15 和 25 M)可以增加 nitrate、cyclic GMP 和 cyclic GMP 相關 vasodilator-stimulated phosphoprotein (VASP) Ser157 的磷酸化。Phorbol-12,13-dibutyrate (150 nM)和 collagen (1 g/ml)可以活化血小板 protein kinase C 活化指標 Mr 47,000 (P47)，CAPE (15 和 25 M)可以明顯的抑制由這兩個活化劑所引起的磷酸化反應。另外，CAPE (15 和 25 M)可以減少由 collagen (1 g/ml)刺激血小板所導致的 ESR 訊息和由 collagen (10 g/ml)所引起 Akt 以及 MAPKs 家族包括 ERK2、JNK1 和 p38 MAPK 的磷酸化反應。

由結果證實，CAPE 抑制血小板活性的作用可能牽涉下列路徑：(一) CAPE 可以抑制 collagen 相關的血小板反應。(二) CAPE 會增加血小板細胞內 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。綜合以上結果，導致 CAPE 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 CAPE 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

Caffeic acid phenethyl ester (CAPE) is an active component of propolis, which is a resinous hive product collected by honeybees from various plant sources. It has been shown to exhibit anticancer, anti-inflammatory and immunomodulatory activities in a broad spectrum of systems. However, the pharmacological affects of CAPE on platelet function are not yet understood, we are interested in investigating the inhibitory effects of CAPE on cellular signal transduction during the process of platelet activation. In this study, CAPE concentration-dependently (6-25 M) inhibited collagen (1 g/ml) induced human platelets aggregation, human platelet-rich plasma and ATP-release reaction without affecting those induced by thrombin (0.01 U/ml), AA (60 M), U46619 (1 M), ADP (20 M) and epinephrine (10 M). The concentration-response curve of collagen induced platelet aggregation was shifted to the right by CAPE (15-100 M) in a concentration dependent manner, the Schild plot showed the pA2 was 4.28, with a slope of -0.826. Convulxin, an agonist of the collagen receptor glycoprotein VI (GPVI), purified from C. durissus terrificus venom and aggretin, a potent platelet activator, was isolated from Calloselasma rhodostoma venom activate platelets by binding to platelet intergrin 2 1. CAPE also inhibited convulxin (100 ng/ml) and aggretin (3.6 g/ml) induced aggregation. Fluorescence from FITC-collagen was attenuated after incubation with CAPE (25 M), indicating that CAPE can compete receptor with collagen. In the presence of CAPE, adhesion of platelets to collagen was diminished in a dose-dependent manner. CAPE (15 and 25 M) inhibited intracellular Ca2+ mobilization, phosphoinositide breakdown, and thromboxane A2 formation stimulated by collagen (1 g/mL) in human platelets. In addition, CAPE (15 and 25 M) markedly increased levels of nitrate、 cyclic GMP and cyclic GMP-induced vasodilator-stimulated phosphoprotein (VASP), Ser157 phosphorylation. Rapid phosphorylation of a platelet protein of Mr 47, 000 (P47), a marker of protein kinase C activation, was triggered by phorbol-12,13-dibutyrate (150 nM) and collagen (1 g/mL). This phosphorylation was markedly inhibited by CAPE (15 and 25 M). Moreover, CAPE (15 and 25 M) reduced the electron spin resonance (ESR) signal intensity of hydroxyl radicals in collagen (1 g/ml)-activated platelets and MAPKs family phosophorylation including ERK2、 JNK1 and p38 MAPK stimulated by collagen (10 g/ml) in human platelets.

In conclusion, our study suggested that the possible pathways of anti-platelet activity of CAPE (15 and 25 M) may involve the following pathway: (1) CAPE blocks collagen-mediated platelet functions such as: adhesion and ATP release reaction. (2) CAPE 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. (3) CAPE significantly inhibited thromboxane A2 formation through reducing the hydroxyl radicals and phosphorylation of MAPK family to inhibit phospholipase A2-cyclooxygenase pathway, and intracellular Ca2+ mobilization. Taken together, CAPE may be used as an effective tool in treating pathological disorder associated with platelet hyperaggregability.