辣椒素 capsaicin 抑制人類大腸癌細胞株 Colo 205 增生並誘發細胞凋亡之機轉

Capsaicin inhibits proliferation of human colon cancer Colo 205 cells via inducing cell cycle arrest and apoptosis

中文摘要

近年來在台灣癌症十大死因中，結腸直腸癌一直高居第三位。辣椒素(Capsaicin)，爲辣椒(Hot Pepper)中主要產生辛辣的成分，許多相關研究指出 capsaicin 具有抗致癌、抗突變及化學防治(chemoprotective)的活性，能引發多種癌症細胞，如:胃癌細胞、前列腺癌細胞及乳癌細胞的凋亡，並具有抑制血管新生的能力。目前一些研究已證明Capsaicin可使癌細胞內產生大量ROS、並使Ca2+由內質網釋放出來，引發癌細胞內凋亡機制啓動。但以 Capsaicin 用於人類大腸癌研究卻很少。所以在此次實驗中，我們利用 capsaicin 與人類大腸癌細胞（Colo 205 cell）作用，藉以探討 capsaicin 對於人類大腸癌細胞 Colo 205 所導致的細胞凋亡（Apoptosis）、細胞週期停滯（Cell cycle arrest）之機制、細胞內蛋白表現、Ca2+的變化)，用以評估 capsaicin 對於大腸癌的抗癌成效。

將 Colo 205 細胞以 Capsaicin 作用後，利用流式細胞儀分析細胞的存活率及細胞內粒線體膜電位(Δ)、Ca2+濃度與 ROS 變化。Real-Time PCR 技術偵測引發細胞凋亡的基因表現。最後以 Western Blot 方法觀察能引發或抑制細胞凋亡的蛋白質之相關性及表現程度。結果發現，作用後 Colo 205 細胞內 p53 大量表現，並誘使 p21 進一步抑制 G1 時期的蛋白激酶複合物活性，造成細胞停滯於 G0/G1 時期(G0/G1 arrest)。同時也觀察到，在 150 μM Capsaicin 作用 24 小時能誘發細胞凋亡之內、外在路徑，造成細胞凋亡。其中細胞內 Caspase-8、-9 及-3 的 mRNA 表現上升，而 Caspase-8、-9 及-3 蛋白質也被活化。

在外在路徑方面，細胞中 Fas、FADD 蛋白表現增加，造成 Caspase-8 及-3 被活化。內在路徑方面發現，細胞內的 ROS、Ca2+產生增加，進而使粒線體上抗凋亡 Bcl-2 家族蛋白表現下降，無法繼續抑制促凋亡 Bcl-2 家族蛋白之活性，最後造成粒線體膜電位改變，Cytochrome c 釋放出粒線體，活化 Caspase-9 及-3，造成細胞凋亡。此外，實驗中觀察到 AIF 蛋白表現增加，可推測除了 Caspase-dependent 凋亡路徑活化外，caspase-independent 凋亡路徑亦被活化。

目前知道有一群統稱爲 IAPs (inhibitor of apoptosis proteins) 的蛋白，可以藉由和 caspase 結合抑制 caspase 活性，進而阻止細胞凋亡。但在實驗中，我們觀察到細胞內 xIAPs 蛋白表現下降，造成 caspase 活性無法被抑制，進而使細胞走向細胞凋亡。

綜合以上結果，Capsaicin 可使 Colo 205 細胞生長停滯於 G0/G1 時期，並同時啓動多條細胞凋亡路徑，造成細胞凋亡。證明其具有抗腫瘤功效，是一種具開發潛

力的抗癌藥物。

英文摘要

In Taiwan, Colorectal cancer is the third most common cancer in men and women. The spicy ingredient in hot pepper was isolated by Thresh , and was named it capsaicin .According to some studies, Capsaicin is an effective component of nature Chinese herb medicine and with a powerful anti-tumor, against mutation and chemoprotective activity. Capsaicin is capable of inducing apoptosis of cancer cells, including gastric cancer, prostate cancer and breast cancer etc. In addition, Capsaicin also inhibits human angiogenesis.Several studies have already shown that Capsaicin could produce a large number of intracellular reactive oxygen species (ROS) and promote that calcium release from endoplasmic reticulum (ER) to damage cancer cells and then cause the activation of apoptosis mechanism in cancer cells. However, there are few studies of the effect of Capsaicin on Colorectal cancer. Because Capsaicin could induce a large of intracellular reactive oxygen species in cancer cells, we will detect the drug-induced DNA damage and the influence on cell cycle process which promotes the proliferation effect on cancer cells. Finally, we will examine the other apoptosis mechanism- endoplasmic reticulum stress (ER stress) associated with the release of intracellular calcium, and then evaluate the inhibition effect of Capsaicin on human Colo 205 cells.

The assays methods are using : 1) flow cytometry for examining the cell cycle arrest and apoptosis; inclusive of cell viability, the levels of ROS, Ca^{2+}, and mitochondrial membrane potential in human Colo 205 cells. 2) Real-Time PCR and flow cytometry to detect mRNA expression. 3)Western blotting methods for examining the apoptosis associated proteins to find out the mechanism of apoptosis. The approach taken in this experiment is to demonstrate that p53 level elevates obviously and causes p21 to inhibit the activity of protein kinase complex , that decreased the percentage of the Colo 205 cells in the S- and G2/M- phases, and increased the percentage in G0/G1-phase (G0/G1 arrest). Based on the result of this study, the extrinsic and Intrinsic pathways of apoptosis were induced. The caspase-3, -8 and -9 mRNA levels increased after treatment with 150μM Capsaicin. The protein of caspase caspase-3, -8 and -9 also be activated after treatment with 150μM Capsaicin .

The extrinsic apoptosis pathway is triggered by Fas ligand (FasL) on account of the amount of Fas and FADD protein elevation. These proteins results in the formation of caspase-8 and caspase-3, which trigger the execution of apoptosis. The percentage of ROS and Ca^{2+} levels are significantly different between Capsaicin treated group and control. We also found the expression of anti-apoptosis Bcl-2 family lower down. Due to anti-apoptosis Bcl-2 not able to inhibit apoptosis in these examined cells,

mitochondria membrane release cytochrome c to cause the activations of Caspase-9、-3 which result in apoptosis.
On account of the elevation of AIF protein, we suggest that both Caspase-Dependent and independent pathways of apoptosis were activated.
So far as we know, a group of inhibitor of apoptosis protein (IAPs) can bind to caspase and block apoptosis. In this study, the decrease of xIAPs lead to apoptosis due to no inhibition of caspase activity.
From the above cited, Capsaicin induced cell cycle arrest of Colo 205 cells in G0/G1-phase and caused apoptosis via extrinsic and Intrinsic pathways. Capsaicin is a strong potential agent for the treatment of Colorectal cancer since it induced apoptosis through the activation of caspase activity in Colo 205 cells.