牡丹皮抑制 A431 癌細胞株轉移及生長作用之評估

Evaluate the inhibitory effects of Paeonia suffruticosa cortex on A431 cell line for growth and metastasis-associated properties

中文摘要

癌症居國內十大死因之首位，其中子宮頸癌對女性而言，更是位居癌症死亡率的第一名，平均約有百分之八十的癌症病人因癌細胞之轉移（入侵性）而致命，故若能有效抑制癌細胞的轉移及入侵，將可大幅減低癌症的死亡率。類黃鹼素 (flavonoids) 廣泛地存在於高等植物之中，一般而言，每人每日從飲食中攝取的類黃鹼素可高達 1 公克或者更多。目前類黃鹼素被發現的生物功能中包括：抑制癌細胞轉移、抑制癌細胞週邊血管增生、誘導細胞凋亡、抗發炎及抗氧化等。此外，關於類黃鹼素在抗癌活性方面的研究，發現類黃鹼素是藉由抑制細胞生長、分化的特定蛋白酶，來達到對抗癌細胞生長的目的。因此若以 flavonoids 來治療病患，或許可以藉由抑制癌細胞分泌 Matrix Metalloprotinases（MMPs），來達到降低癌細胞轉移的發生。本研究證實了牡丹皮的成份 quercetin 及 kaempferol，對人類子宮頸上皮細胞癌—A431 細胞株，顯示其抑制癌細胞生長與分泌 MMPs 之效果。

首先牡丹皮以水萃取，再以 EtOAc 及 BuOH 劃分後，接著 EtOAc 層部分以 Sephadex LH-20 管柱層析法，以酒精和水作梯度沖提分離後，可得到 Fraction 1?6，而 Fraction 5 的部分則表現出最佳的癌細胞活性抑制能力。之後 Fraction 5 再以 ODS 管柱層析法，以甲醇和水作梯度沖提，經分離純化得到二個類黃鹼素—quercetin 和 kaempferol。兩者顯示出明顯的抑制 A431 子宮頸癌細胞株生長（其 IC50 分別爲 12 μg/mL、18 μg/mL）及分泌 MMPs 的能力（8 μg/mL、16 μg/mL）。另外由 DNA 光學顯微鏡攝影和 DNA fragment 萃取電泳分析實驗中，也可以觀察到它們確實可以誘導癌細胞進行細胞凋亡（程式性死亡），產生 DNA 片斷和染色體斷裂凝縮的現象。綜合以上結果，我們推論 quercetin 及 kaempferol，在抗癌藥物的發展上深具潛力。

英文摘要

Cancer is the top of ten causes of death in Taiwan, especially cervical cancer threatens all of the female. The metastasis (invasion) of cancer cells leads 80% patients died, and inhibition of cancer cells metastasis will markly decrease the death rate. The flavonoids are present in almost all higher plants, in the normal diet they were consumed more than one gram per day. Flavonoids display a wide range of pharmacological properties including anti-metastatic, anti-cacinogenic, anti-inflammatory and anti-oxidant effects. Recent studies have demonstrated that flavonoids are inhibitors of several protein kinases involved in signal transduction. Treatment with flavonoids may inhibit secretion of these MMPs in tumor cells, and thereby reduce the potential for metastasis. In this study, we evaluated the effects

of Paeonia suffruticosa constituents on human epidermoid carcinoma cells A431 proliferation, and matrix metalloproteinases (MMPs) secretion.
Dried cortex of Paeonia suffruticosa were extracted with water. The water extract was divided into EtOAc and BuOH soluble fraction. The EtOAc soluble fraction was separated by gel filtraction （Sephadex LH-20, EtOH：H_2O） to afford fractions Ⅰ－Ⅵ. Fraction Ⅴ showed potential inhibitory effects. Then it was chromatographed on ODS column with MeOH and H_2O by gradient elution to afford quercetin and kaempferol. Both of them significantly inhibited A431 cells proliferation （IC50 12 μg/mL、18 μg/mL） and MMPs secretion （8 μg/mL、16 μg/mL） respectively. It was also found that they could induce cell morphological change and apoptosis （the programmed cell death as indicated by DNA fragments in electrophonic analysis）. This study supports that kaempferol and quercetin may have potential as anti-cancer and anti-metastasis agents.