萃取物之生體外免疫調節功能評估

In Vitro Assement of Immunomodulating Factor from Extract of Propolis

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[摘要]

蜂膠(Propolis)是由蜜蜂蜂巢所得來的，東方國家將蜂膠當作民間醫療使用。本研究採用市售蜂膠乙醇萃取物(ethanol extract of propolis, EEP)及其活性成分 Caffeicacid phenethyl ester (CAPE)進行生體外免疫調節功能活性之評估。EEP 和 CAPE 各別在 0.25 mg 及 3.0 g 濃度下具有抑制人類子宮頸癌細胞株 (HeLa cells) 生長效果；EEP 及 CAPE 均能降低白血病細胞株 (Leukemia cell lines)如 HL-60、U-937 的存活率。EEP 於 0.5 mg/mL，CAPE 於濃度 6.25 g/mL 均顯現癌細胞的 DNA 片段化，此抑制效果於細胞上出現皺縮及凋亡體之形態學特徵上的改變。以 10 mM TPA 活化人類單核球細胞株(THP-1)分化為巨噬細胞，CAPE 於 10 g/mL 濃度時，THP-1 細胞株吞噬 E. coli bacteria 的能力為 7.66%，增加至 20 g/mL 時，降為 4.05%，顯示 CAPE 可以抑制 TPA 所誘導之發炎狀態，故可降低 THP-1 之吞 噬活性。小鼠脾臟細胞當加入 CAPE 12.5g 劑量時，可增加殺死小鼠淋巴癌細胞株(YAC- 1)的數目，增加自然殺手細胞(NK cells)活性能力達 3.42%，並隨著劑量的增加，NK 活性也 愈大；EEP 0.5 mg 濃度可增加 55.44% NK 活性，顯示 Propolis 能增強小鼠免疫力。EEP 作用 於老鼠巨噬細胞 J774.1，在濃度 0.25 mg/mL 下，具有抑制 LPS 1g/mL 併用 IFN-γ10 U/ mL 所誘導之 NO 釋出量；對另一株老鼠巨噬細胞 RAW264.7，於濃度 0.5 mg/mL 時亦具有抑制 NO 的釋出。CAPE 於濃度 6.25 g/mL 即有抑制老鼠巨噬細胞株 J774.1 之 iNOS 蛋白催化活性，當濃度高達 25g/mL 則達到完全抑制效果。此外，以 TPA 誘導人類乳癌細胞株(MCF-7、MDA- MB-453)產生之 COX-2，CAPE 於 20 g/mL 劑量亦具有抑

制 COX-2 蛋白表現作用。進一步 以 RT-PCR 確認 J774.1 細胞在 EEP 濃度 6.25 g/mL，可完全抑制其基因的表現。而 CAPE 於濃度 1.25M 時亦可抑制 iNOS 基因表現。另以 LPS 併用 IFN-γ 誘導老鼠巨噬細胞株 J774.1，應用生物晶片做成 cDNA 微矩陣(microarry)研究，分析其基因表現情形，結果顯示 J774.1 細胞株有 510 個基因有表現，285 個基因上調(upregulatory)，225 個基因下調(downregulatory)。此外，以 TPA 誘導人類乳癌細胞株(MCF-7)，使用 8000 個人類 cDNA 微 矩陣雜交，結果顯示 MCF-7 細胞株有 279 個基因有表現，215 個基因上調，64 個基因下調。這些 cDNA 微矩陣結合生物資訊 (bioinfor-matics) 能對調節基因之重要性及影響層面，提供更深一層的訊息。

關鍵字: 蜂膠 Propolis、蜂膠乙醇萃取物 EEP CAPE、氧化氮 NO
誘導型氧化氮合成酶 iNOS、 第二型環氧酶 COX-2
生物晶片 Biochip

[摘要]

Propolis obtained from honeybee hives has been used in Oriental folkmedicine. Caffeic acid phenethyl ester (CAPE) is an active ingredient isolated from propolis. The present study, assessment the effect of ethanol extract of propolis (EEP) and CAPE that possesses anti-cancer, anti-inflammatory, and immunomodulatory activity. EEP was analysed for the determination of total flavonoid content by using the aluminium nitrate method, UVspectrophotometry. EEP contained high total flavonoid content. It was observed EEP showed possessed stronger DPPH free radical scavenging activity. We was foundthat EEP and CAPE entered leukemia cells and then inhibited their survival in a concentration-dependent manner. EEP induced characteristic DNAfragmentation and morphological changes typical of apoptosis in these cells. Theseresults suggest that EEP is a potent apoptosis-inducing agent in

human leukemiacells. EEP and CAPE signifycantly inhibited NO production and iNOS proteinexpression induced by lipopolysaccharide (LPS) plussing interferon gamma. EEP andCAPE also signifycantly inhibited COX-2 protein expression induced by Phorbol myristate acetate (TPA). These results suggest that EEP and CAPE may exertits anti-inflammatory effect by inhibiting the iNOS gene expression and by directly inhibiting the protein activity of iNOS and COX-2. Finally,analysis of gene expression at the RNA level using cDNA against an array of 15000mice genes showed that 510 (3.4%) of the genes were expressed in the J774.1cells. A total of 285 genes (1.9 %) were found to be upregulated and 225 genes(1.5%) were found to be downregulated. Analysis of gene expression at the RNAlevel using cDNA against an array of 8000 human genes showed that 279 (3.5%) ofthe genes were expressed in the MCF-7 cells. A total of 215 genes (2.7%) were found to be upregulated and 64 genes (0.8%) were found to bedownregulated. This using of cDNA microarry coupled to bioinformations can providein-depth information on the impact and importance of the regulated genes.