多酚類天然物之抗癌活性

Antitumor Activity of Natural Occurring Polyphenols

中文摘要

「癌」為目前威脅人類健康重要疾病之一，而臨床使用之抗癌藥物已發現有多種抗藥性及副作用產生，因此抗癌藥的研究，顯得日趨迫切需求；而由中藥材及天然物中有系統的進行安全、有效抗癌藥物篩檢，即為新藥探索的重要途徑之一。本研究應用體外癌細胞株毒性建立一快速篩選模式，首先應用口腔上皮癌細胞(KB)進行體外細胞毒性篩選，並進行疾病導向之癌細胞株檢測，具有細胞抑制活性之天然物再進行體內動物抗癌試驗。並利用流式細胞儀(flow cytometry)分析藥物對細胞分裂周期的影響；及細胞攝入(incorporation) 3H 取代的 thymidine, uridine 與 leucine 的影響，探求是否具有抑制 DNA, RNA 與蛋白質生合成作用；觀察藥物對癌細胞造成毒性後，細胞形態變化 (光學顯微鏡及電子顯微鏡)；再利用分子生物分析法，如 agarose gel electrophoresis 、Western blot 等電泳分析，探討其引起細胞毒性之作用機轉。

36 種水解型單寧（含單體 11 個、二量體 21 個及多量體 4 個），對體外癌細胞活性之抑制作用，結果顯示二量體之單寧具有較明顯之癌細胞生長抑制；其中以 tellimagrandin II 及 geraniin 鍵結型之二量體 (euphorbins G, F) 其抑制作用比 1,2,3,4,6-Penta-O-galloyl-b-D-glucopyranose (PGG)及 geraniin 鍵結型之二量體 (euphorbins A, B)強且大環型之二量體皆具明顯抑制癌細胞生長作用。然而多量體分子量增加其細胞毒性並不會隨之增強，顯示單寧之體外抗癌活性與其官能基之結構有特異性，其中以大環型之二量體最具活性。.

二量體大環水解型單寧 cuphiin D1 、cuphiin D2、oenothein B、及 woodfordin C，皆具有抑制 KB、肝癌細胞(Hep 3B)、子宮頸癌細胞(HeLa)、攝護腺癌細胞(DU-145)、淋巴癌細胞(HL-60)等細胞生長，且對正常羊膜細胞(WISH)及肝細胞(Chang Normal Liver)抑制作用較小。故將具有體外癌細胞抑制活性作用之 cuphiin D1 、cuphiin D2、oenothein B、及 woodfordin C 皮下注射予 S-180 擔癌鼷鼠，結果四個大環類單寧皆對前三天注射藥物組，顯示有意義延長生命作用；且會刺激健康人單核細胞釋放 IL-1b，證實其體內抗癌作用，屬 host-mediated antitumor 作用。其中以 cuphiin D1 抗癌作用最強，故進一步探討其抑制癌細胞活性之作用機轉。結果發現 cuphiin D1 會引起 HL-60 細胞的 DNA 斷鏈並沉積於細胞核周圍，且會抑制 Bcl-2 蛋白表現，顯示 cuphiin D1 誘導 HL-60 細胞進行凋零(apoptosis)式死亡。綜合上述結果，cuphiin D1 在體內、外試驗中均呈明顯抗癌活性，是 36 個測試單寧中最具開發潛力之天然物。

英文摘要

Cancer has been reported as the important disease cause of death in human. However

many drugs of chemotherapy caused resistance that has been reported in clinical studies. Therefore, it is very necessary to develop new antitumor drugs. Chinese medicines and natural products have been widely used in traditional therapy, that are good sources to be developed as new antitumor drugs by a well screening system. In our screening system, we initially used KB cell line to screen the cytotoxic effects of natural products by MTT assay, and then continued to use another tumor cell line. If the compound has antitumor effects with in vitro system, we further explored in vivo antitumor effects and the mechanism of its antitumor effects. Moreover we used flow cytometry, incorporation of radioactive precursors into macromolecules, agarose gel electrophoresis, Western blot, the alteration of cellular morphology and other methods to study its mechanism of causing cell death.

In this study, we screened 36 kinds of ellagitannins in vitro. The results showed that tellimagrandin II-geraniin type dimers (euphorbins G, F) were more cytotoxic than PGG-geraniin type dimers (euphorbins A, B). All of the macrocyclic hydrolyzable tannins such as cuphiin D1 (KB, IC50=20.0ug/ml), cuphiin D2 (KB, IC50=20.7ug/ml), oenothein B (KB, IC50=26.8ug/ml), and woodfordin C (KB, IC50=28.9ug/ml) showed potent effects. However, trimeric and tetrameric ellagitannins did not increase the cytotoxicity index. The results suggested the antitumor effects of tannin need certain ellagitannin monomer units.

We evaluated the antitumor activities of four macrocyclic hydrolyzable tannin dimers, cuphiins D1, D2, oenothein B and woodfordin C. All the macrocyclic dimer tannins significantly inhibited the growth of the human carcinoma cell lines KB, HeLa, DU-145, Hep 3B, and the leukemia cell line HL-60. The macrocyclic dimer tannins showed less cytotoxicity than adriamycin against normal cell lines (WISH and Chang normal Liver). All four compounds inhibited the viability of S-180 tumor cells in an in vitro assay and an in vivo S-180 tumor-bearing ICR mice model. Furthermore, in the in vitro treatment of mononuclear cells from human, cuphiins D1, D2, oenothein B and woodfordin C stimulated release of an IL-1b from cells. The results suggest that cuphiin D1 and woodfordin C exert their antitumor effects through potentiation of host-immune defense via activation of macrophages and cuphiin D1 was the most potential than the others. However, HL-60 cells treated with 30ug/ml cuphiin D1 for 36h exhibited chromatin condensation, a marker for apoptosis by electron micrographs. Moreover, HL-60 cells treated with cuphiin D1 for 36h showed a dose-dependent decrease in cell viability and the uptake of 3H-thymidine, -uridine, -leucine. Flow cytometric analysis demonstrated apoptotic cells with low DNA content, a decrease of G2/M cells and a concomitant increase of G1 cells. Cuphiin D1 also caused DNA fragmentation and inhibited Bcl-2 expression in the cells, detected by electrophoresis. Taken together, these results suggested that inhibition Bcl-2 expression in HL-60 cells might account for the mechanism of cuphiin D1-induced apoptosis. In summary, cuphiin D1 has antitumor effects in vitro and in vivo and may have potential for antitumor applications.