Magnolol 抑制癌細胞之分子機轉研究

Studies one the molecular mechanisms of anti-cancer effect of magnolol

中文摘要

Magnolol 是由中國傳統中藥厚朴中所分離出來含有 hydroxylated biphenyl 化合物的，本研究的目的為探討其抗癌作用及其可能的作用分子機轉。本研究的結果顯示，雖然在非常低的濃度下(3-10 mM) Magnolol對於人類大腸癌細胞(COLO-205)及肝癌細胞(Hep-G2)的 DNA 合成及數目增生具有抑制作用，且其抑制作用與 Magnolol的劑量呈正向相關的方式。然而 Magnolol的這些抑制作用則不見於人類的正常細胞如表皮細胞(keratinocytes)、纖維母細胞(fibroblasts)及人類臍靜脈內皮細胞(HUVEC)。Magnolol 在此濃度下(3-10 mM)對這些人類癌細胞並不具毒性。此結果顯示 magnolol對於癌細胞生長的抑制作用是經由抑制細胞週期。利用[3H]thymidine incorporation 及流式細胞儀(flow cytometry)的分析，我們發現 magnolol可抑制癌細胞 DNA 的合成，且使細胞週期停滯在 G0/G1 期。利用西方點墨法分析(Western blot analysis)及酵素測定(kinase assay)，我們進一步推測 magnolol可能是藉由增加抑制細胞週期的調控蛋白 p21Cip1 的表現，而抑制 cylin-CDK 複合物的活性，進而使細胞的週期停滯於 G0/G1，而造成增生的抑制。

當進一步探討 magnolol引起 p21Cip1 蛋白表現的分子機轉我們發現 magnolol可造成 COLO-205 細胞內鈣離子濃度上升的現象。而這些由 magnolol引發的細胞質內鈣離子增加可引發 protein kinase c (PKC)蛋白的活化。PKC 是屬於 serine-threonine 酵素的家族。以前的報告曾指出 PKC 的活化與大腸癌致癌機轉有關。當進一步探討在 COLO-205 細胞中 PKC 訊息傳導途徑是否參與 magnolol所引發之 p21Cip1 蛋白表現增加，我們發現在投與 magnolol前以 PKC 抑制劑(staurosporine)對 COLO-205 細胞作預先處理，可防止因 magnolol所引發之 p21Cip1 蛋白表現增加。另外，我們也發現以 magnolol處理 COLO-205 細胞後，可造成 PKCg 由細胞質轉位至細胞膜，顯示對 PKCg 的活性具有促進作用。此外，根據以下的研究證據，包括：(a) Extracellular signal-regular kinase (ERK) 及 c-Jun NH2-terminal kinase (JNK)二者皆被 magnolol所活化以及(b)將 COLO-205 細胞以 MEK (mitogen-activated protein kinase kinase)抑制劑(PD98059)作預先處理可防止因 magnolol所引發之 p21Cip1 蛋白表現增加，我們進一步推測 ERK 及 JNK 所調控的訊息傳導路徑皆可能參與了 magnolol在 COLO-205 細胞中所引發之 p21Cip1 蛋白表現增加的現象。

當我們將 magnolol的濃度增加至 100 mM 時，36 小時的處理下 COLO-205 及 Hep-G2 細胞則產生凋亡現象(apoptosis)，但人類正常牙齦纖維母細胞(fibroblasts)及臍靜脈內皮細胞(HUVEC)對相同的處理則無此反應。接著我們

利用 Hep-G2 細胞來當作探討 magnolol引發細胞凋亡的分子機轉模式。100 mM magnolol處理後在 Hep-G2 細胞內產生了以下的一些變化：(a) 細胞質內鈣離子濃度增加，(b) 由粒腺體至細胞質之轉位的細胞色質 c (cytochrome c) 量增加，(c) caspases 3, 8, 9 被活化，及(d) bcl-2 蛋白的表現被抑制。若以 phospholipase C 抑制劑，
1-[6-[[(17b)-3-methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-1H-pyrrole-2,5-dione (U73122)，或細胞內鈣鰲合劑，
1,2-bis(2-aminophenoxy)ethane-N,N,N'',N''-tetraacetic acid acetoxymethylester (BAPTA/AM) ，作預處理，則 magnolol在Hep-G2細胞引發的細胞質內鈣離子增加的量及caspases 8與 9 的活化程度被會被抑制，同時magnolol所引發之 Hep-G2 細胞的凋亡現象也有明顯的減少。利用對抗Fas的抗體 (ZB-4)來處理Hep-G2細胞，則magnolol所引發之 caspases 8 活化的程度會受到抑制，同時 Hep-G2 細胞的凋亡現象也有明顯的減少。綜合以上的發現，我們推測細胞質內鈣離子濃度的增加、細胞色質 c由粒線體內位移至細胞質、及 Fas 所引發之訊息傳遞途徑等，皆參予了 magnolol所引發的 Hep-G2 細胞凋亡之分子機轉。
最後以裸鼠當作動物模式，來測試magnolol在生物體內之抗癌作用。首先我們將 COLO-205 細胞植入裸鼠的皮下組織中使之產生腫瘤，再由裸鼠腹膜腔中每日注射給與magnolol(100 mg/kg) 。兩個星期的藥物處理後，裸鼠皮下的腫瘤生長受到明顯的抑制，抑制效果高達 85% 。在這些生長受抑制的腫瘤組織中，可觀察到p21Cip1 蛋白表現量增加，以及細胞凋亡的現象。重要的是，如此的藥物處理並未對裸鼠產生明顯的毒性反應。綜合以上的研究結果，我們首次證實了magnolol在活體外及活體內皆具有抑制腫瘤細胞生長的能力，也闡述了其可能的作用分子機轉。

英文摘要

Magnolol, a hydroxylated biphenyl compound isolated from the Chinese herb Hou p''u of Magnolia officinalis, has been reported to have anti-cancer activity. The purpose of this study is to evaluate the anti-cancer effect of magnolol and its possible molecular mechanisms. In this study, magnolol at very low concentrations of 3-10 mM inhibited DNA synthesis and decreased cell number in cultured human cancer cells (COLO-205 and Hep-G2) in a dose-dependent manner, but not in human untransformed cells such as keratinocytes, fibroblasts, and human umbilical vein endothelial cells (HUVEC). Magnolol was not cytotoxic at these concentrations and this indicates that it may have an inhibitory effect on cell proliferation in the subcultured cancer cell lines. [3H]thymidine incorporation and flow cytometry analyses revealed that magnolol treatment decreased DNA synthesis and arrested the cells at the G0/G1 phase of the cell cycle. Moreover, the magnolol induced cell cycle

arrest occurred when the cyclin-CDK system was inhibited just as p21Cip1 protein expression was augmented.

We further evaluated the molecular mechanisms of magnolol-induced p21Cip1 protein upregulation and found that magnolol dose-dependently increased intracellular calcium concentration in COLO-205 cells. The increased intracellular calcium concentration can further activate protein kinase C (PKC). PKC is a family of serine-threonine kinases that has been implicated in the regulation of colon tumorigenesis. Pretreatment of COLO-205 cells with a PKC inhibitor (staurosporine) prevented the magnolol-induced upregulation of p21Cip1 protein, suggesting that PKC pathways was involved in the magnolol-induced expression of p21Cip1 protein. Furthermore, treatment of COLO-205 cells with magnolol resulted in PKCg isoform translocation from cytosol to membrane, indicating that PKCg was activated by magnolol. Based on the findings of the present study including that (a) both extracellular signal-regular kinase (ERK) and c-Jun NH2-terminal kinase (JNK) were activated in the magnolol-treated COLO-205 cells; (b) pretreatment of MEK (mitogen-activated protein kinase kinase) inhibitor (PD98059) blocked the magnolol-induced p21Cip1 in COLO-205 cells, we conclude that both ERK and JNK pathways are involved in the magnolol-induced upregulation of p21Cip1 protein in COLO-205 cells.

When magnolol concentration was increased to 100 mM, apoptosis was observed in COLO-205 and Hep-G2 cells after 36 h of magnolol treatment, but not in human untransformed gingival fibroblasts and HUVEC. For delineation of molecular mechanisms of magnolol-induced malignant cell apoptosis, Hep-G2 cells were used as a model. Treatment of Hep-G2 cells with 100 mM magnolol showed a sequence of associated intracellular events which included: (a) increased cytosolic free Ca2+, (b) increased translocation of Cyto c (cytochrome c) from mitochondria to cytosol, (c) activation of caspases 3, 8 and 9, (d) downregulation of bcl-2 protein. Pretreatment of the cells with phospholipase C inhibitor, 1-[6-[[(17b)-3-methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-1 H-pyrrole-2,5-dione (U73122), or intracellular chelator of Ca2+, 1,2-bis(2-aminophenoxy)ethane-N,N,N'',N''-tetraacetic acid acetoxymethyl ester (BAPTA/AM), inhibited the subsequent magnolol-induced increase of [Ca2+]i, and also the activation of caspases 8 and 9 so that the occurrence of apoptosis in those cells was greatly reduced. Pretreatment of the cells with ZB4 (which disrupts the Fas response mechanism) also decreased the subsequent magnolol-induced caspase 8 activation and reduced the occurrence of apoptosis. We interpret these findings to indicate that the above listed sequence of intracellular events lead to the apoptosis observed in Hep-G2 cells, and that [Ca2+]i, Cyto c, and

Fas function as intracellular signals to coordinate those events.
Finally, we used nude mice as an animal model to study the anti-cancer effect of magnolol in vivo. COLO-205 cells implanted subcutaneously in nude mice formed solid tumors; subsequent daily intraperitoneal injections of magnolol (100 mg/kg) led to profound regression of these tumors of up to 85%. In these tumors, an increase in the expression of p21Cip1 protein level and the occurrence of apoptosis were observed. Importantly, there was no significant toxicity to nude mice under this condition. These findings demonstrate for the first time that magnolol can inhibit the proliferation of tumor cells in vitro and in vivo. In this study, we also delineated the possible molecular mechanisms of the anti-cancer action of magnolol.