Magnolol 對人類肝癌細胞之抗肝癌活性的機制研究

Studies on the Mechanisms of Magnolol-Induced Anti-tumoral activity in Human Hepatoma Cells

中文摘要

本篇論文主要在探討中藥厚朴的酚類萃取物 magnolol 對人類肝癌細胞株(Hep G2)生長的抑制與其作用機制。厚朴是一種民間常用的中藥材，廣泛的用於治療發燒 、頭痛、焦慮、腹瀉及中風等病症。厚朴酚(magnolol)是從厚朴中萃取而來的酚類化合物。而根據我們研究的結果顯示，magnolol 在低濃度(10~50 μM)時會對肝癌細胞株的生長產生抑制的作用，並有細胞週期停滯(cell cycle arrest)的現象。而高濃度的 magnolol (100 μM)則會造成細胞凋亡(apoptosis)的發生。但是對一般普通細胞如人類纖維母細胞(human fibroblast)及人類臍靜脈內皮細胞 -Human Umbilical Vein Endothelial Cell (HUVEC) 卻不會有影響。在進一步了解 magnolol 造成這些作用的機轉時，發現 magnolol 所引起的細胞週期停滯(cell cycle arrest)與 cyclin dependent kinase (CDK) inhibitor 如 p21 的表現量增加有關。而在細胞凋亡(apoptosis)的部分則發現除了高濃度 magnolol (100 μM)會造成 Hep-G2 細胞在 72 小時發生 DNA fragmentation 與 sub G0/G1 peak 的現象外，若將培養液的牛胎兒血清濃度降低到 1%，則使細胞凋亡的現象提前在藥物處理四小時後便出現。在同樣的時間，caspase 8 也有明顯的切割情況發生。另外 Fas receptor，一種引發細胞凋亡的重要膜蛋白，在肝癌細胞株上的表現也直接影響了由 magnolol 所引發的細胞凋亡。我們發現會表現 Fas receptor 的肝癌細胞株對於 magnolol 有較高的感受性。利用 Fas antagonist antibody 對 Fas receptor 作 neutralization 可部分阻斷由 magnolol 所造成的細胞凋亡現象及部分回復 casepase 8 的切割。

本研究的結果顯示，magnolol 在低濃度會造成肝癌細胞株的生長抑制，而在高濃度則會引發細胞的凋亡現象。對於 magnolol 的抗癌作用機轉的了解，相信會有助於此藥物在臨床的使用。

英文摘要

The objective of this thesis research is to investigate the anti-tumoral action of magnolol on the hepatoma cell line Hep-G2 and its underlying molecular mechanisms. In vitro studies demonstrated that magnolol at low concentrations (10~50 μM) induced a dose-dependent inhibition of Hep-G2 growth and cell cycle arrest. On the other hand, magnolol at a higher concentration (100 μM) induced an apoptosis of Hep-G2 cells. However, magnolol at these concentrations had no effect on human untransformed cells, such as human fibroblasts and human umbilical venous endothelial cells (HUVEC). Western blot analysis demonstrates that an increased expression of cyclin-dependent kinase (CDK) inhibitor, p21, is the major factor contributing the magnolol-mediated cell cycle arrest. Treatment of Hep-G2 with 100 μM magnolol caused DNA fragmentation and sub-G0/G1 peak at 72 hours when the

cell was grown in the culture medium containing 10 % fetal bovine serum. Several apoptosis-related proteins including caspase 8 were shown to be activated in response to magnolol treatment in Hep-G2. Administration of Fas antagonist antibody (ZB4), which neutralized the Fas receptor, prevented the magnolol-mediated caspase 8 cleavage and suppressed the magnolol-induced apoptosis. This result suggests that Fas receptor may be involved, at least partially, in the magnolol-induced apoptosis. In conclusion, our data demonstrated that magnolol at low concentrations (10-50 μM) induced an anti-proliferation of Hep-G2 and at a higher concentration (100 μM) caused apoptosis. Only when the mechanism of its effect is fully understood can we begin to design a strategy to use magnolol for anti-cancer therapy.