Terfenadine 誘發人類癌細胞 G0/G1 週期停滯與凋亡之分子機制探討

Studies on the Molecular Mechanisms of Terfenadine - induced Apoptosis and G0/G1 Cell Cycle Arrest in Human Cancer Cells

中文摘要

Terfenadine－－第二代 H1 組織胺的拮抗劑，僅對周圍末梢神經 H1 受體有選擇性之阻斷作用，對中樞神經系統無鎮靜或其他抑制作用，在臨床上原來是用於治療過敏的藥物。我們將人類癌細胞處理 Terfenadine 隨著處理劑量與時間的增加，細胞的存活度降低，生長也有被抑制，利用流式細胞儀分析發現 Terfenadine 可以使人類癌細胞的細胞週期停滯於 G0/G1 時期，並且爲不可逆的反應。觀察調控細胞週期蛋白含量，我們發現 p21、p27 表現量上升，CyclinD1、CyclinD3、CyclinB、CDK2、CDK4 表現量減少。經 kinase assay 測定發現 CDK2、CDK4 活性明顯下降。經過瓊膠電泳分析 Terfenadine 會使人類癌細胞產生 DNA ladder 的特徵，表示細胞發生凋亡，並隨著劑量的增加我們發現：p21、p27、Bax、Bad 表現量上升，而 PARP 有被切斷的片段產生，Bcl-2、Caspase-3 減少，而且 p27 有被切斷的情形產生。合併使用比單獨處理 Ketoconazol 或 Terfenadine 人類癌細胞 SubG1 的含量與 DNA ledder 有增加的現象，其中又以 HepG2 細胞最爲明顯，Terfenadine 主要透過 Cytochrome P450 3A4 代謝，HepG2 在合併處理藥物後觀察到 Cytochrome P450 3A4 蛋白表現量明顯下降。綜合以上結果，Terfneadine 除了具有原本的抗組織胺效果外，在本實驗也發現具有另外新的功能，可將細胞週期停滯並導致細胞凋亡，另外當它與 Ketoconazole 合併使用時發現會增強 Terfenadine 的作用。

英文摘要

Terfenadine is the second generation of H1-antagonists Non-sedating antihistamines that have been used in the treatment of allergic rhino conjunctivitis. In clinical trials, terfenadine has demonstrated a lesser degree of sedation than first-generation H1 receptor antagonists. In our study, we found that human cancer cell growth rate were inhibited by terfenadine treatment. In order to investigate the mechanisms of terfenadine-induced cell growth arrest, human cells treated with terfenadine and the response of genes that regulated cell cycle were determined. Western blot analysis revealed the terfenadine results in an induction of the p21、p27，and a down-regulation of Cyclin D1、CyclinB、CDK2、CDK4. The terfenadine treatment also resulted in decrease in kinase activities associated CDK2、CDK4. We also found that apoptosis was induced by terfenadine in human cancer cell by DNA laddering formation. In this study，we demonstrated that p21、p27、BAX and BAD protein were induced，in contrast，Bcl-2 protein was down regulated by treated with terfenadine. Interestingly,

we demonstrated that the percentage of SubG1 and DNA ladder were strongly potentiated in human cancer cell when combine treated with ketoconazol and terfenadine. Such results indicate that except the original function of terfenadine， terfenadine have some other new effects resulted in induce of human cancer cell apoptosis, inhibition of cell growth， and G0/G1 phase arrest.