建立探討細胞週期及細胞凋亡之新模型

Establishment of new model system to investigate cell cycle and apoptosis

中文摘要

Miconazole 是全世界臨床上使用的口服抗黴菌藥。此論文是第一篇經研究證實 Miconazole 能使癌細胞停滯在 G0/G1 時期，進而抑制癌細胞的生長。由過去的研究結果指出，不同的癌症細胞，其 p53 的表現會有些許差異。藉由流式細胞儀的分析以及細胞生長曲線的結果，我們觀察到由 Miconazole 誘發的癌細胞生長週期停滯的效果，在 COLO 205 和 Hep G2 細胞(具有 wild-type p53)比 HT 29 (p53 His273 突變)、HL60（p53 null）及 Hep 3B 細胞(缺少 p53 基因)要好。我們認為 p53 的表現及經由 p53 調控的細胞生長週期路徑和 Miconazole 所引發的癌細胞 G0/G1 時期停滯的過程有強烈的相關性。同時，Miconazole 會引起大腸癌細胞株 p53，p21，p27 等蛋白質表現量增加，且抑制 Cyclin D1、D3 及 CDK4 等蛋白質的表現，而且 Miconazole 還會經由活化 caspase（s）誘發細胞凋亡的發生。由以上結果，我們證實了 Miconazole 誘發人類惡性癌細胞生長週期停滯及凋亡的分子機制，所以我們認為 Miconazole 具備的這些效果，使其成為具有潛力的癌症治療藥物。
Podophyllotoxin 是目前用來治療尖形濕疣的主要藥物，在 1940 年以前曾用來嘗試治療癌症，但因其毒性所以慢慢被禁用。本研究是就此藥會對微小管造成分解作用進而探討 Podophyllotoxin 對癌細胞的細胞週期和凋亡的分子作用機轉。我們的研究顯示，相同的劑量（2mM）可造成 HT29 百分之九十以上的細胞停滯在 G2/M 期，但是對 HL60 卻是促進細胞凋亡。進而研究其機轉，發現 PD 會使 HT29 細胞內的 MPF 活性上升，而在 HL60 細胞則可以在短時間（六到十二小時）引發 Bcl-2 的磷酸化、caspases 的活化，最後造成細胞凋亡。
因此我們認為 Podophyllotoxin 可用在 HT29 細胞研究 G2/M 週期停滯，或是用在 HL60 細胞來探討 Bcl-2 磷酸化的機轉的絕佳工具。而且目前臨床上常發生單獨使用一種化療藥物時容易使癌細胞出現抗藥性而導致療效降低，所以將 PD 與其他抗癌藥物併用預期應會有良好的效果。

英文摘要

In this study, we have demonstrated that miconazole, a widely used oral-antifungal agent, can inhibit cell cycle progression in G0/G1 phase and is a potent inhibitor of cyclin dependent kinase (CDK4). DNA fragmentation analysis revealed that apoptosis was induced in a dose dependent manner by miconazole treatment. The caspase 3 was activated and its specific substrate, poly（ADP-ribose）polymerase, was degraded at 18-24 h after miconazole treatment. Dose-dependent experiment was performed and demonstrated that the bax gene expression was elevated. In this study, we used

different type of human cancer cells with various p53 status to investigate the mechanisms of miconazole-induced G0/G1 arrest. Our results demonstrated that miconazole-treated cells had an approximately 6-fold increased in the expression of p21 and p27. Whereas Cyclin D3 and cyclin D1 were down regulated in a dose- and time-dependent manner. Moreover, the expression of PCNA, cdk2, and cyclin E levels were not significant change when compared with untreated cells. CDK4 but not CDK2, activity immunoprecipitated from cells treated with miconazole was markedly inhibited. Concomitantly, hypophosphorylation form of the Rb protein was detected in samples treated with miconazole.

In this study, we have demonstrated that apoptosis was easily induced by treatment with a low dose (0.02mM) of podophyllotoxin (PD) in HL60 cells. However, from the flow cytometry and western analysis we found that the same dose of PD resulted in induction of G2/M cell cycle arrest in HT29 cells. Immunofluoresence staining analysis revealed that the PD-induced mitotic arrest is due to microtubule degradation. PD-induced G2/M arrest were characterized by (a) induction of abnormal mitotic spindle formation, (b) elevation of cyclin B1/cdc2 and cdk7 kinase activity, and (c) down-regulation of Wee-1 protein expression. On the other hand, caspase 3、8 and 9 activation, Bcl-2 hyperphosphorylation and the release of cytochrome c from mitochondria were demonstrated to be involved in PD-induced apoptosis. Taken together, these results suggest that PD could be used in combination with other anticancer drugs in chemotherapy.