黑殭菌素(Destruxin B)對DBA/2老鼠L5178Y淋巴癌細胞之抑制效果及其機制

The Anti-Tumor Effects of Destruxin B on L5178Y Lymphoma Cell

中文摘要

黑殭菌素(Destruxin)是由昆蟲寄生性真菌(entomogenous fungi)之一的黑殭菌(Metarhizium)所分泌出的毒素，在已分離出的三十餘種中，本研究中採用的是Destruxin B(DB)作爲抗腫瘤製劑。經發酵後自發酵液中抽取純化後，先以毛細管電泳及質譜儀鑑定其純度，純化出的 DB 以 Acetonitrile 爲溶劑配製。在本實驗中，我們採用 DBA/2 品系老鼠經 methylcholanthrene 所引導出的 L5178Y 淋巴癌細胞作反應。分別用 in vitro 及 in vivo 的模式觀察到 DB 對於對腫瘤細胞之生長影響。結果中發現 1.29 μM 及 2.58 μM 的 DB 對腫瘤細胞生長有顯著的抑制作用，當劑量超過 5.17 μM 時有毒殺腫瘤細胞的作用。然而相同劑量的 DB 對正常脾臟細胞及纖維母細胞株則沒有抑制作用。利用流式細胞儀觀測藥物引發細胞週期停滯於 G2/M 時期，並進一步誘使其發生細胞凋亡的現象。此外，也探討了會影響細胞週期行進和調控細胞凋亡的相關蛋白表現，了解到一些 DB 作用下 L5178Y 細胞的分子機轉。在 DB 的刺激下，L5178Y 細胞株的 CDK1(cdc2)蛋白表現會降低、p53 蛋白增加、caspase-3 蛋白被活化，使得細胞週期停滯並且會發生細胞凋亡。最後再以 DBA/2 品系老鼠作 in vivo 試驗，藉由建立攜有 L5178Y 淋巴癌細胞的 DBA/2 品系小鼠爲動物模式，以腹腔注射方式投予 DB，觀察到 DB 可以延緩小鼠死亡的時間點，證實了其在體內抗癌的效果。本實驗冀望爲未來抗癌藥物開展一個新的領域。

英文摘要

Destruxin is a substance which secrete from entoenous fungi, Metarhizium. This substance is toxic to insect and has been used as an insecticide for decades. Over thirty species of destruxins have been isolated from different investigators. In this study, the purified destruxin B (DB) was tested for its in vitro and in vivo anti-tumor activities by using L5178Y lymphoma cells which was induced by methylcholanthrene from T lymphocytes of DBA/2 mice. The results indicated that DB suppresses L5178Y lymphoma growth dramatically even in the dose as low as 1.29 μM . When doses of DB over 5.17 μM , the dead tumor cells were found.The same doses range has no growth suppression or tumoricidal effect on either mouse spleen cells primary culture or NIH3T3 fibroblast cells. The results from flow cytometric analyses indicated that DB induced cell cycle

arrest at G2/M phase, consequently, caused cell apoptosis. The further elucidate the mechanisms of apoptosis, some regulatory or signal proteins for cell cycle and apoptosis were analyzed by Western blot. The results indicated that under DB treatment, the CDK1 (cdc2) protein expression was suppressed, p53 protein was increased and caspase-3 was activated.

These results confirmed the previous growth suppression and flow cytometric results. The in vivo experiment was performed by implanting L5178Y lymphoma cells into DBA/2 mouse peritoneal cavity i.p. at dose of 230 μg/kg. The results showed that the treated group of mice my extent their survival days at least for 2 weeks longer than that of control group.

From both in vitro and in vivo experiment results, it has proven that DB may be as a potential candidate for future anti-tumor drug.