Loratadine 抑制人類直腸腫瘤細胞(COLO 205)生長之分子機制研究

Studies of the antitumor Effects of Loratadine on Human Colon Carcinoma

中文摘要

本篇研究計畫的主題是探討抗組織胺(antihistamine) Loratadine (LOR)對人類大腸癌細胞株(COLO205)的生長抑制作用及其作用機轉。LOR 是一種長效型的抗組織胺，臨床上用此藥來治療過敏性鼻炎，包括：打噴嚏、流鼻水、眼睛癢等症狀，是屬於 H1-receptor antagonist。

先前並沒有任何研究指出具 LOR 有抑制癌細胞生長效果。本研究發現 LOR 會抑制大腸癌細胞 COLO205 的生長。將不同劑量的 LOR 作用於 COLO205 細胞 24 小時，可觀察到 25 uM LOR 會使細胞產生細胞凋亡(apoptosis)的現象，而 50 uM LOR 則會造成細胞週期 G2/M 停滯的現象。以西方墨點法進一步分析 apoptosis 相關蛋白表現，發現 caspase 8、caspase 3、bid、caspase 9 、PARP 有被活化的現象；此外，cytochrome C 及 AIF 也分別從粒腺體中釋出到細胞質及細胞核中。而在 LOR 調控之 COLO205 細胞週期 G2/M 時期停滯現象中，也發現 p53 及 p21Cip1 表現增加，CDK2、cyclinA2、cdc2、cyclinB 表現減少，Cdk2-cyclinA kinase 活性下降。進一步探討 25 uM 與 50 uM LOR 所造成的細胞生長抑制作用當中，發現 Myt1 活性下降，cdc25c 活性上升，並促使 cdc2-cyclinB kinase 活性上升。但相對於 25 uM ，50 uM LOR 則會使 cdc2-cyclinB kinase 的活性顯著下降，推測可能是 LOR 造成細胞週期 G2/M 時期停滯的主因。

英文摘要

In the present study, we have demonstrated the molecular mechanisms of antitumor effects of Loratadine (LOR) on human colon adenocarcinoma cells (COLO205). LOR is considered to be a long-acting antihistamine with selective peripheral histamine H1-receptor agonistic activity which has been used to relieve allergic rhinitis (seasonal allergy) symptoms, including sneezing, runny nose, itching, and watery eyes.

There were none have previously been shown that LOR can inhibit the growth of human Carcinoma. Here, we provide evidence that LOR has antitumor effect on human colon cancer cell line (COLO205). After 24 hours of treatment with dose-dependent LOR, evidence for induction of apoptosis (25 uM)and cell cycle G2/M arrest (50 uM) were clearly documented by several assays including flow cytometry cell cycle analysis, DNA laddering. The levels of apoptosis and cell cycle regulatory protein were determined by Western blot analysis. Treatment of 25 uM

LOR induced apoptosis by activation of caspase 8, caspase 3, bid, caspase 9, and PARP. Moreover, cytochrome C was released from mitochondria into the cytoplasm, and AIF was translocated from mitochondria to the nucleus. Clearly, LOR-induced cell apoptosis involved in both death receptor and mitochondria pathway. By 50 uM LOR inducing cell cycle G2/M arrest, the protein levels of p53 and p21Cip1 were significantly elevated, but the expression of CDK2、cyclinA2、cdc2、cyclinB were decreased as well as the inhibition of Cdk2-cyclinA kinase activity. However, overactivation of Cdc2-cyclinB kinase significantly occurred with 25 uM and 50 uM LOR treatment by phosphorylation of cdc25c and inactivation of Myt1, but Cdc2-cyclinB kinase activity was down regulated when treated LOR 50 uM compared with 25 uM These results suggested that down regulation of Cdc2-cyclinB kinase activity was the major reason of G2/M arrest