一種新型 IkappaB Kinase 抑制劑 Zerumbone

抑制前發炎物質生成之機轉探討

Mechanisms of Zerumbone, a Novel IkappaB Kinase Inhibitor, Suppressed Proinflammatory Mediators Production in Human Pulmonary Epithelial Cells

中文摘要

一、 本論文主要探討亞熱帶生薑的倍半帖成分 Zerumbone 抑制人類肺臟上皮細胞(A549)受 interleukin-1beta(IL-1b)引發之 cyclooxygenase-2(COX-2)表現及 IL-6、IL-8 釋放之機制研究。

二、 細胞前處理 Zerumbone(10-50 microM)以濃度相關的方式抑制 IL-1b 引發 COX-2 表現，IL-6 的釋放、IL-8-luciferase 的活性及 IL-8 的釋放。進一步實驗證實 Zerumbone(10-50 microM)也依濃度相關方式抑制 kB-luciferase 的活性及 NF-kB-特異性 DNA-蛋白複合物的形成。IL-1b 引發 p65 及 p50 從細胞質位轉至細胞核的作用也會受到 Zerumbone(10-50 microM)所抑制。Zerumbone(10-50 microM)會抑制 IL-1b 所誘導 p65 Ser536 的磷酸化，卻不會抑制 p65 Ser276 磷酸化。同樣地，Zerumbone 也會抑制 IkappaB 在細胞質中的磷酸化及降解現象。進一步的實驗證實，由 IL-1b 所誘導的 IKK alpha/beta 磷酸化及活性同樣地也可以被 Zerumbone 所抑制。

三、 利用 in vitro IKK kinase assay，發現 Zerumbone 直接抑制 IKK 激?的活性。然而，Zerumbone 並不會影響 IL-1b 所引發的 NIK、p44/42 MAPK 及 p38 MAPK 的活化。此外，Zerumbone 也會抑制 IL-1b 所誘導 AP-1 與 DNA 結合的能力及 AP-1-luciferase 的活性。IL-1b 會依時間相關的方式誘導 c-jun 和 c-fos 蛋白的表現，此反應則會被 Zerumbone、Bay117082(IkappaB 磷酸化抑制劑)及 NF-kappaB inhibitor peptide 所抑制。然而，Zerumbone 並不會影響 IL-1b 所引發的 c-jun 磷酸化及 JNK 的活化。

四、 綜合以上的結果顯示，在 A549 細胞中，Zerumbone 可抑制 IL-1b 誘導的發炎物質產生，且經由抑制 IKK 蛋白激?的活性而來。因此，Zerumbone 是一個新的 IKK 的抑制劑可發展一個有效抑制肺部發炎反應的藥物。

英文摘要

1. In this project, we undertaken to explore the action mechanism of zerumbone, a sesquiterpene found in subtropical ginger,suppresses interleukin-1beta (IL-1b)-induced cyclooxygenase-2 expression, IL-6 and IL-8 release in human pulmonary epithelial cells (A549).

2. Pretreatment of cells with zerumbone (10-50 microM) attenuated IL-1b-induced

increase in COX-2 expression, IL-6 release, IL-8-luciferase activity and IL-8 release via a concentration dependent manner. Moreover, Zerumbone (10-50 microM) inhibited the IL-1b-induced increase in kB-luciferase activity and NF-kB-specific DNA-protein complex formation in a concentration-dependent manner. The IL-1b-induced translocation of p65 and p50 from the cytosol to the nucleus was inhibited by zerumbone (10-50 microM). Zerumbone (10-50 microM) inhibited the IL-1b-induced p65 phosphorylation at Ser536, but not at Ser276. Similarly, zerumbone (10-50 microM) also inhibited IL-1b-induced IkappaB phosphorylation and degradation in the cytosol fraction. Furthermore, the IL-1b-mediated increases in IKKalpha/beta phosphorylation and IKKalpha/beta activity were also inhibited by zerumbone.

3. Using in vitro IKK kinase assay, we found that zerumbone directly inhibited the IKKalpha/beta kinase activity. However, zerumbone did not affect the IL-1b-induced activations of NF-kappaB-inducing kinase (NIK), p44/42 mitogen-activated protein kinase (MAPK), and p38 MAPK. In addition, zerumbone inhibited IL-1b-induced increase in the formation of AP-1-specific DNA-protein complex and the AP-1-luciferase activity. Treatment of IL-1b caused the induction of c-jun and c-fos protein in a time-dependent manner, and these effects were inhibited by zerumbone, Bay 117082 (an IkappaB phosphorylation inhibitor), and an NF-kappaB inhibitor pepetide. However, these inhibitors had no effect on IL-1b-induced c-jun phosphorylation and c-jun N-terminal kinase (JNK) activation.

4. Taken together, we demonstrate that zerumbone inhibits IL-1b-induced proinflammatory protein production in A549 cells; this inhibition is mediated by suppressing IKK enzyme activity. Our results suggest that zerumbone is a novel IKK inhibitor and may be an effective anti-inflammatory drug.