GSNO 引起人類大腸癌細胞凋亡之分子機制探討

Molecular Mechanisms of GSNO-induced Apoptosis in Human Colon Cancer Cells

中文摘要

ＧＳＮＯ(S-nitrosoglutathione) 爲細胞內抗氧化物ＧＳＨ攜帶一氧化氮之化合物，當它釋出一氧化氮時，可對細胞造成ＤＮＡ傷害。已有許多報告指出一氧化氮自由基可造成細胞凋亡，但ＧＳＮＯ引起細胞凋亡之詳細機制尚未被研究清楚。在本實驗中，我們使用人類腸癌細胞株，來探討ＧＳＮＯ對細胞之毒性及細胞凋亡之機制。

細胞凋亡(Apoptosis)爲一種細胞自殺性之死亡方式，其細胞型態的變化包括細胞膜形成皰狀、染色質濃縮及 Apoptotic bodies 之形成等等；本實驗我們依型態變化、TUNEL Assay 及ＤＮＡ段片形成(ＤＮＡ fragmentation) 等現象來判斷發現以ＧＳＮＯ處理腸癌細胞株(ＨＴ29，Colo205) 確實會引起細胞凋亡。我們亦發現，細胞給予ＧＳＮＯ處理之同時，若一起加入銅離子(Cu＋＋)，則細胞死亡率較單獨處理ＧＳＮＯ明顯減少。據此我們亦證實了 Cu＋＋在細胞外可促使ＧＳＮＯ釋出ＮＯｏ，由於ＮＯｏ不穩定，在極短時間內轉變爲硝酸鹽及亞硝酸鹽，因而降低進入細胞內ＮＯｏ之量，而減少細胞的傷害。

ＮＯｏ調控細胞凋亡過程中之基因表現，是我們想要嘗試了解的，以西方墨點分析來偵測基因表現的變化，結果發現 Bad、Bax 及 c-Jun 等蛋白的表現皆有增加之現象，而 p27 及 Bcl-2 的表現卻有被抑制的現象。爲了解ＮＯｏ在參與細胞內訊息傳遞所扮演之角色，我們也對 PKA 及 PKC 之表現進行探討，結果發現 PKA 與 PKCζ之表現會被ＮＯｏ抑制，此結果告訴我們在ＧＳＮＯ引起之細胞凋亡中，存在一種特殊的調節機制，而 PKA 及 PKCζ可能扮演一重要角色。

英文摘要

GSNO(S-nitrosoglutathione) is an intracellular NO donor. According to previous studies, NO induced apoptosis was demonstrated. However, the detail mechanism of apoptosis induced by GSNO is not yet understood. In this report, GSNO synthesized in vitro was used to study the mechanism of cellular toxicity and apoptosis in human colon adenocarcinoma cell lines. Apoptosis is cells executed through a suicide program that show

distinctive morphological changes, including membrane blebbing, chromatin condensation, and formation of apoptotic bodies. We realized that apoptosis was induced by GSNO in HT29 and Colo205 cell lines, by examination of morphological changes, Tunel Assay, and DNA ladder. We also found that the mortality rate in cells treated by GSNO was obviously decreased when simultaneous treated with copper ions. We suggest that Cu++ promote extracellular NO release from GSNO and thus leading to decrease intracellular NO exposure. Because NO is rapidly converted to nitrate and nitrite in the solution phase, measurement of nitrite can serve as an indirect method to evaluate NO production. In our study, we demonstrated that GSNO was decomposed in the presence of Cu++ by Nitric Oxide Assay.

We wondered whether some proteins which expression induced by GSNO could modulate the apoptosis. To test this hypothesis, we treated the cells with GSNO in a time-dependent manner, and detected protein expression by using western blot. In this study, we demonstrated that the Bad and Bax protein were induced, in contrast, p27 and Bcl-2 protein were inhibited in HT29 cells treated by GSNO. On the other hand, induction of c-Jun by GSNO was also observed in a time-dependent manner. In order to investigate the roles of NO involve in signal transduction in HT29 cells. We also studied the relationship between NO-induced apoptosis and PKA or PKC protein expression. We found that the isoenzyme of PKC--PKCζ, and PKA were down regulated during apoptosis. Our results show that a specific modulatory mechanism was observed in GSNO-induced apoptosis. The PKCζ and PKA may play an important role

in this process.