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论著

去甲斑蝥素诱导人卵巢癌SK-OV-3细胞发生有丝分裂期阻滞与凋亡

李瑞婧，洪兴福，季媛媛，孙震晓

北京中医药大学中药学院生物制药系，北京 100102

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摘要 目的 考察去甲斑蝥素(NCTD)对人卵巢癌SK-OV-3细胞生长的抑制作用，探究其诱导细胞发生有丝分裂期阻滞及凋亡的过程及相关性。方法 NCTD 30，60，120和240 μmol·L^{-1}分别作用人卵巢SK-OV-3细胞24，48和72 h后，MTT法检测细胞存活率；NCTD 60 μmol·L^{-1}分别作用SK-OV-3细胞0，6，12和24 h后，流式细胞术测定细胞周期的变化；NCTD 60 μmol·L^{-1}分别作用SK-OV-3细胞0，6，12和24 h，倒置显微镜下检测其对细胞形态学变化的影响；Giemsa染色检测细胞核的变化；间接免疫荧光术结合激光扫描共聚焦显微镜技术检测细胞内微管分布及有丝分裂期纺锤体形成的影响。NCTD 60 μmol·L^{-1}分别作用12和36 h后，流式细胞术检测细胞凋亡情况；再将NCTD 60 μmol·L^{-1}作用12 h后的细胞，采用温和的机械振荡法分离为悬浮细胞和贴壁细胞，继续作用24 h，流式细胞术及Giemsa染色检测分析两个时相两种细胞凋亡的影响。结果 MTT结果显示，NCTD 30~240μmol·L^{-1}对SK-OV-3细胞的生长抑制作用存在明显的时间(P<0.05)和浓度依赖性(P<0.05)；NCTD作用SK-OV-3细胞24，48和72 h的IC_{50}分别为(261.3±2.4)μmol·L^{-1}，(48.3±1.7)μmol·L^{-1}和(10.9±1.0)μmol·L^{-1}。NCTD 60 μmol·L^{-1}分别作用于SK-OV-3细胞0，6，12和24 h，SK-OV-3细胞逐渐表现出G_2/M期阻滞，呈时间依赖性；倒置相差显微镜下观察，NCTD引起SK-OV-3细胞逐渐收缩变圆，与周围细胞分离；Giemsa染色可见处于有丝分裂期的细胞显著增多，多个核细胞比例增多；免疫荧光染色发现，NCTD组细胞的微管系统受到干扰，有丝分裂期细胞纺锤体形成异常。NCTD作用细胞12和36 h凋亡率分别达20.4%和62.3%；将NCTD作用12 h的细胞，人工振荡分离为悬浮细胞和贴壁细胞，检测凋亡情况，同时检测继续作用24 h后两组细胞凋亡情况，发现处于有丝分裂间期的SK-OV-3细胞凋亡率大于处于有丝分裂期的细胞。结论 NCTD主要通过诱导人卵巢癌SK-OV-3细胞G_2/M期阻滞和细胞凋亡抑制细胞生长。干扰细胞有丝分裂过程中微管的组装和纺锤体的形成可能是NCTD诱导SK-OV-3细胞M期阻滞的原因之一。NCTD诱导的SK-OV-3细胞发生凋亡可不依赖细胞M期阻滞。

关键词 去甲斑蝥素 人卵巢癌 SK-OV-3细胞 细胞分裂 微管 纺锤体 细胞凋亡

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Norcantharidin induced mitotic arrest and apoptosis in human ovarian cancer SK-OV-3 cells

LI Rui-jing, HONG Xing-fu, JI Yuan-yuan, SUN Zhen-xiao

Department of Biopharmaceutics, College of Chinese Pharmacy,Beijing University of Chinese Medicine, Beijing 100102, China

Abstract

OBJECTIVE To investigate the mechanisms of mitotic arrest and apoptosis of human ovarian cancer SK-OV-3 cells induced by norcantharidin (NCTD). **METHODS** Cell viability was detected by MTT assay after cells treated with NCTD 30, 60, 120 and 240 μmol·L^{-1} for 24, 48, and 72 h, respectively. Cell cycle and apoptosis were assayed through flow cytometry after cells treated with NCTD 60 μmol·L^{-1} for 0, 6, 12 and 24 h, respectively. Morphological changes in SK-OV-3 cells with NCTD 60 μmol·L^{-1} treated for 0, 6, 12 and 24 h were observed under microscope, respectively. The morphological changes in cell nuclei were observed after Giemsa staining. Effects of NCTD on microtubule organization and spindle formation in SK-OV-3 cells were detected and analyzed by indirect immunofluorescence staining and laser scanning confocal microscope. To investigate the relationship between apoptosis and SK-OV-3 cells induced by NCTD 60μmol·L^{-1} treated for 12 and 36 h, the cells were separated to adherent and non-adherent cells by gently physical shaking after NCTD was treated for 12 h and cells in 2 groups were continually treated with NCTD for 24 h, then analyzed by FCM and Giemsa staining. **RESULTS** Cell viability obviously decreased in NCTD 30-240 μmol·L^{-1} groups in a dose-dependent (P<0.05) and time-dependent manner (P<0.05). The IC_{50} of NCTD in SK-OV-3 cells treated for 24, 48 and 72 h were (261.3±2.4)μmol·L^{-1}, (48.3±1.7)μmol·L^{-1} and (10.9±1.0)μmol·L^{-1}, respectively. After SK-OV-3 cells were treated with NCTD 60 μmol·L^{-1} for 0, 6, 12 and 24 h, G_2/M phase cells accumulated in a time-dependent manner. Some cells showed cellular shrinkage and were separated from other cells and M phase cells increased in NCTD group.

Immunostaining indicated that normal microtubule system of cells and mitotic spindle assembly were damaged in NCTD groups. In addition, the NCTD resulted in high cell apoptosis rate, which was 20.4% and 62.3% at 12 and 36 h. Analysis of apoptosis of adherent and non-adherent cells of NCTD treated for 36 h showed that apoptosis increased in interphase, but decreased in M phase . **CONCLUSION** NCTD can induce G_2/M phase arrest and apoptosis in human ovarian cancer SK-OV-3 cells. Interfering microtubule organization and spindle formation during cell cycle progression is part of the mechanisms of M phase arrest in NCTD treated SK-OV-3 cells. Apoptosis induced by NCTD does not rely on M phase arrest in SK-OV-3 cells.

Key words norcantharidin human ovarian cancer SK-OV-3 cell cell division microtubule spindle apoptosis

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通讯作者 孙震晓,Tel:010-84738646,E-mail:sunzxcn@hotmail.com sunzxcn@hotmail.com