Paclitaxel induces apoptosis in human gastric carcinoma cells

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Received: 2002-08-03  Accepted: 2002-11-21

Abstract

AIM: To investigate the apoptosis in gastric cancer cells induced by paclitaxel, and the relation between this apoptosis and expression of Bcl-2 and Bax.

METHODS: In in vitro experiments, MTT assay was used to determine the cell growth inhibitory rate. Transmission electron microscope and TUNEL staining method were used to quantitatively and qualitatively detect the apoptosis status of gastric cancer cell line SGC-7901 before and after the paclitaxel treatment. Immunohistochemical staining was used to detect the expression of apoptosis-regulated gene Bcl-2 and Bax.

RESULTS: Paclitaxel inhibited the growth of gastric cancer cell line SGC-7901 in a dose-and time-dependent manner. Paclitaxel induced SGC-7901 cells to undergo apoptosis with typically apoptotic characteristics, including morphological changes of chromatin condensation, chromatin crescent formation, nucleus fragmentation and apoptotic body formation. Paclitaxel could reduce the expression of apoptosis-regulated gene Bcl-2, and improve the expression of apoptosis-regulated gene Bax.

CONCLUSION: Paclitaxel is able to induce the apoptosis in gastric cancer. This apoptosis may be mediated by down-expression of apoptosis-regulated gene Bcl-2 and up-expression of apoptosis-regulated gene Bax.

Zhou HB, Zhu JR. Paclitaxel induces apoptosis in human gastric carcinoma cells. World J Gastroenterol 2003; 9(3): 442-445

http://www.wjgnet.com/1007-9327/9/442.htm

INTRODUCTION

Apoptosis is a form of cell death characterized by active cellular suicide during T-cell clonal deletion, embryogenesis, and DNA damage. Apoptotic cell death is often associated with distinctive characteristics, such as nuclear fragmentation, cytoplasmic blebbing, and internucleosomal fragmentation of DNA. Whether a cell committed to apoptosis partly depends upon the balance between proteins that mediate cell death, such as Bax, and proteins that promote cell viability, such as Bcl-2 or Bcl-xl. Overexpression of Bax has been shown to accelerate the cell death. Overexpression of antiapoptotic proteins such as Bcl-2 represses the death function of Bax. Thus, the ratio of Bcl-2 to Bax appears to be a critical determinant of a cell’s threshold for undergoing apoptosis.

Microtubule inhibitors such as paclitaxel can increase tubulin polymerization, tubulin bundling, and cell cycle arrest. Paclitaxel have been proven to induce apoptosis in many cancers. In order to study the mechanism of paclitaxel induces apoptosis of SGC-7901 gastric cancer cells, MTT assay was used to determine the cell growth inhibitory rate. Transmission electron microscope and TUNEL staining method were used to quantitatively and qualitatively detect the apoptosis status of gastric cancer cell line SGC-7901 before and after the paclitaxel treatment. Immunohistochemical staining was used to detect the expression of apoptosis-regulated gene Bcl-2 and Bax.

We report here the results of our findings showing paclitaxel inhibited the growth of gastric cancer cell line SGC-7901 in a dose-and time-dependent manner. Paclitaxel induced SGC-7901 cells to undergo apoptosis with typically apoptotic characteristics, including morphological changes of chromatin condensation, chromatin crescent formation, nucleus fragmentation and apoptotic body formation. Paclitaxel reduces Bcl-2 expression and improves Bax expression on SGC-7901 cells.

MATERIALS AND METHODS

Materials
Paclitaxel was obtained from Xiehe Pharmaceutical Factory in Beijing. MTT was obtained from Sigma Chemical Co. Ltd. Anti-Bcl-2 monoclonal antibody and anti-Bax monoclonal antibody were purchased from Beijing Zhongshan biotechnology Co. Ltd.

Methods
Cell culture Human gastric carcinoma cell line SGC-7901 was obtained from laboratory in Shandong Provincial Hospital and maintained in RPMI 1640 supplemented with 100 ml·L⁻¹ fetal bovine serum, 100 kU·L⁻¹ penicillin, 100 mg·L⁻¹ streptomycin and 2 µmol·L⁻¹-L-glutamine under 50 ml·L⁻¹ CO₂ in a humidified incubator at 37 °C. SGC-7901 cells were incubated for different time periods in the presence of paclitaxel at 0.001, 0.01, 0.1, 1 µmol·L⁻¹.

MTT assay 1x10⁵ cells/well in a 96-well plate after 24 hours incubation were treated with increasing concentrations of paclitaxel (0.001 µmol·L⁻¹ to 1 µmol·L⁻¹) for 24 to 96 hours. 10 µL of 5 g·L⁻¹ of MTT was added to the cells in every well and incubated for 4 hours at 37 °C. Culture media were discarded followed by addition of 0.2 ml of DMSO and vibration for 10 minutes. The absorbance (OD) was measured at 570 nm using a microplate reader. The cell growth inhibitory rate was calculated as follows: (OD of control group - OD of experimental group)/OD of control group×100 %.

Transmission electron microscopy The cells treated with 0.1 µmol·L⁻¹ paclitaxel were trypsinized and harvested after 24 hours. Subsequently the cells were fixed in 4 % glutaral and immersed with Epon 821, imbedded in capsules and converged for 72 hours at 60 °C. The cells were prepared into ultrathin section (60 nm) and stained with uranyl acetate and lead citrate. Cell morphology was examined by transmission electron microscopy.

TUNEL assay Apoptosis of SGC-7901 cells was evaluated by using an in situ cell detection kit (Beijing Zhongshan biotechnology Co. Ltd). The cells were treated in the presence or absence of 0.1 µmol·L⁻¹ paclitaxel for 24 to 96 hours and fixed in ice-cold 80 % ethanol for up to 24 hours, treated with proteinase K and then 0.3 % H₂O₂, labeled with fluorescein...
dUTP in a humid box for 1 hour at 37 °C. The cells were then combined with POD-Horseradish peroxidase, colorized with DAB. Controls consisted of omission of fluorescein dUTP. Cells were visualized with light microscope. The Apoptotic Index (AI) was calculated as follows: AI=(Number of apoptotic cells/Total number)×100%.

**Immunohistochemical staining** Immunohistochemical staining was accomplished utilizing an avidin-biotin technique. SGC-7901 cells treated in the presence or absence of 0.1 μmol·L⁻¹ paclitaxel for 24 to 96 hours were grown on six-well glass slides and fixed in acetone. After washing in PBS, the cells were incubated in 0.3% H₂O₂ solution at room temperature for 5 minutes. The cells then were incubated with anti-Bcl-2 or anti-Bax at a 1:300 dilution at 4 °C overnight. Following washing in PBS, the second antibody, biotinylated antirat Ig G, was added and the cells were incubated at room temperature for 1 hour. After washing in PBS, ABC compound was added and then incubated at room temperature for 10 minutes. DAB was used as the chromagen. After ten minutes, the brown color signifying the presence of antigen bound to antibodies was detected by light microscopy and photographed at ×200. Controls consisted of omission of the primary antibody. The Positive Rate (PR) was calculated as follows: PR=(Number of positive cells/Total number)×100%.

**Statistical analysis** Data were analyzed employing the paired two-tailed Student t test, and significance was assumed at P<0.05.

**RESULTS**

**MTT assay**

SGC-7901 cells were exposed to increasing concentrations (0.001 μmol·L⁻¹ to 1 μmol·L⁻¹) of commercially available paclitaxel for 24 to 96 hours. Our results show a dose- and time-dependent increase in tumor cell mortality. The data were summarised in Table 1.

**Table 1** The inhibitory effect of paclitaxel on SGC-7901 cells (inhibitory rate, %)

| Time(h) | RPMI-1640 | Paclitaxel (mmol·L⁻¹) | 0.1 | 1 | 10 | 100 |
|---------|-----------|----------------------|-----|---|----|-----|
| 24      | 0.0       | 9.6⁶  | 18.8⁶  | 22.8⁶  | 34.3⁶ |
| 48      | 0.0       | 17.5⁶  | 21.6⁶  | 36.4⁶  | 45.4⁶ |
| 72      | 0.0       | 23.8⁶  | 37.3⁶  | 47.6⁶  | 58.9⁶ |
| 96      | 0.0       | 35.6⁶  | 44.7⁶  | 57.6⁶  | 87.8⁶ |

¹P<0.01, ²P<0.001 vs the control group.

**Figure 1** Paclitaxel-induced apoptosis in SGC-7901 cells with Transmission Electron Microscope. Apoptotic cell with chromatin condensation, chromatin crescent formation, nucleus fragmentation (×4000).

**Morphological changes**

After treatment of SGC-7901 cells with paclitaxel (0.1 μmol·L⁻¹) for 24 hours, some cells appeared apoptotic characteristics including chromatin condensation, chromatin crescent formation, nucleus fragmentation and apoptotic body formation were seen by transmission electron microscope (Figure 1).

**TUNEL assay**

Positive staining located in the nucleus (Figure 2). The results showed after treatment of SGC-7901 cells with paclitaxel (0.1 μmol·L⁻¹) for 24 to 96 hours, the AIs were apparently increased with treat time (P<0.05) (Table 2).

**Figure 2** Apoptotic cells induced by paclitaxel with TUNEL assay (×200)

**Table 2** Apoptotic Index (AI) of treated SGC-7901 cells by paclitaxel

| Time(h) | AI(%)   |
|---------|---------|
| 0       | 1.43±2.42 |
| 24      | 4.89±2.63 |
| 48      | 16.34±1.85 |
| 72      | 27.86±3.23 |
| 96      | 36.49±3.95 |

¹P<0.05, ²P<0.01 vs the control group.

**Expression of Bcl-2 proteins**

Positive staining located in the cytoplasm. The results showed after treatment of SGC-7901 cells with paclitaxel (0.1 μmol·L⁻¹) for 24 to 96 hours, the PRs of Bcl-2 proteins were apparently reduced with treat time (P<0.05) (Table 3). This suggested paclitaxel could reduce Bcl-2 expression.

**Table 3** Positive Rate of Bcl-2 on treated SGC-7901 cells by paclitaxel

| Time(h) | PT(%) |
|---------|-------|
| 0       | 35.44±3.86 |
| 24      | 20.50±2.71 |
| 48      | 10.66±2.36 |
| 72      | 6.78±1.65 |
| 96      | 3.98±1.34 |

¹P<0.05, ²P<0.01 vs the control group.

**Expression of bax proteins**

Positive staining located in the cytoplasm. The results showed after treatment of SGC-7901 cells with paclitaxel (0.1 μmol·L⁻¹) for 24 to 96 hours, the PRs of Bax proteins were apparently improved with treat time (P<0.05) (Table 4). This suggested paclitaxel could improve Bcl-2 expression.
DISCUSSION

Apoptosis is a form of cell death characterized by active cellular suicide during T-cell clonal deletion, embrogenesis, and DNA damage. Apoptotic cell death is often associated with distinctive characteristics, such as nuclear fragmentation, cytoplasmic blebbing, and internucleosomal fragmentation of DNA.[1-5]. The Bcl-2 family plays a central role in the control of apoptosis. This family includes a number of proteins which have amino acid sequence homology, including anti-apoptotic members such as Bcl-2 and Bcl-xL, as well as pro-apoptotic members including Bax and Bad.[7-10]. Overexpression of Bax has been shown to accelerate the cell death[11-15]. Conversely, Overexpression of antiapoptotic proteins such as Bcl-2 represses the death function of Bax.[16-20]. Thus, the ratio of Bcl-2 to Bax appears to be a critical determinant of a cell’s threshold for undergoing apoptosis.[21].

Microtubule inhibitors such as paclitaxel can increase tubulin polymerization, tubulin bundling, and cell cycle arrest.[22-24]. Paclitaxel have been proven to induce apoptosis in many cancers.[25-27]. In order to study the mechanism of paclitaxel induces apoptosis of SGC-7901gastric cancer cells, MTT assay was used to determine the cell growth inhibitory rate. Transmission electron microscope and TUNEL staining method were used to quantitatively and qualitatively detect the apoptosis status of SGC-7901 gastric cancer cell before and after the paclitaxel treatment. Immunohistochemical staining was used to detect the expression of apoptosis-regulated gene Bcl-2 and Bax.

In the present study, MTT assay was used to observe the effect of paclitaxel on the growth of SGC-7901 gastric carcinoma cells in vitro, indicating that the drug could inhibit the the growth of gastric carcinoma cells. Our results show a dose- and time-dependent increase in tumor cell mortality. Its concentration- and time-effect relationships were significant. TUNEL assay showed after treatment of SGC-7901 cells with paclitaxel (0.1 μmol·L⁻¹) for 24 to 96 hours, the AIs were apparently increased with treat time (P<0.05). Immunohistochemical staining was used to detect the expression of Bcl-2 proteins and Bax proteins. The results showed after treatment of SGC-7901 cells with paclitaxel (0.1 μmol·L⁻¹) for 24 to 96 hours, the PRs of Bcl-2 proteins were apparently reduced with treat time (P<0.05), but the PRs of Bax proteins were apparently improved with treat time (P<0.05). These suggested paclitaxel could reduce Bcl-2 expression and improve Bcl-2 expression. The ratio of Bcl-2 to Bax was decreased. The decreased ratio could trigger the apoptosis of SGC-7901 cells.

The research in our laboratory demonstrated paclitaxel is able to induce the apoptosis in gastric cancer. This apoptosis may be mediated by down-expression of apoptosis-regulated gene Bcl-2 and up-expression of apoptosis-regulated gene Bax. The mechanism of Paclitaxel as a chemotherapeutic drug in anti-gastric carcinoma chemotherapy should be further studied.

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Edited by Ren SY