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Original Article

Cytotoxic effects of β-carboline alkaloids on human gastric cancer SGC-7901 cells

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Abstract: To investigate the cytotoxic effects of β-carboline alkaloids on human gastric cancer SGC-7901 cells. Human gastric cancer SGC-790s1 cells were treated with β-carboline alkaloids at the concentration of 0, 10, 20, 30 and 40 μg/ml for 48 hr. Cell viability was measured by Cell Counting Kit-8 assay. Cell apoptosis was detected by Hoechst 33258 staining and DNA fragmentation analysis. The expression of phosphatase and tensin homolog (PTEN) and extracellular signal-regulated kinase (ERK) was examined by quantitative real-time PCR (qRT-PCR) assay and western blot analysis. β-carboline alkaloids inhibited the growth of SGC-7901 cells concentration dependently. β-carboline alkaloids treated SGC-7901 cells displayed apoptotic nuclei as detected using Hoechst 33258 staining. β-carboline alkaloids also induced DNA ladder, indicative of apoptosis in SGC-7901 cells concentration-dependently. Furthermore, β-carboline alkaloids increased PTEN and decreased ERK mRNA expression in SGC-7901 cells in a concentration dependent manner. They also increased PTEN and decreased ERK protein expression. β-carboline alkaloids inhibit the growth and induce apoptosis of SGC-7901 cells. The cytotoxic effects of β-carboline alkaloids might correlate with increased PTEN expression and decreased ERK expression in SGC-7901 cells.

Keywords: β-carboline alkaloids, human SGC-7901 cells, apoptosis, PTEN, ERK

Introduction

β-carboline alkaloids are extracted from the seeds of Peganum harmala in Xinjiang. They have a broad spectrum of anticancer activity and little toxic side effects [1-5]. The crude extract of Peganum harmala has been used in clinical treatment of cancer. However, little is known about its anti-tumor mechanism.

Phosphatase and tensin homolog (PTEN) was first cloned in 1997. It has dual specificity phosphatase activit and turns out to be the first tumor suppressor to have phosphatase activity. PTEN plays important roles in cell apoptosis, cell cycle block, cell migration, etc. PTEN is expressed in a variety of tumors including gastric cancer, cervical cancer, ovarian cancer, laryngeal cancer, etc. [6-9], and is associated with prognosis. Extracellular signal-regulated kinase (ERK) is a member of the mitogen-activated protein kinases (MAPK) family. It participates in cell proliferation, differentiation, morphology, apoptosis, etc. [10]. ERK is overexpressed in many human malignancies [11, 12]. Nowadays, there is little report on the effects of β-carboline alkaloids on the expression of PTEN and ERK.

In this study, the cytotoxic effects of β-carboline alkaloids on human gastric cancer SGC-7901 cells were investigated. β-carboline alkaloids inhibited the growth and induced apoptosis of SGC-7901 cells. It also increased PTEN mRNA and protein expression while decreased ERK mRNA and protein expression in SGC-7901 cells.

Materials and methods

Reagents and cells

β-carboline alkaloids were kindly provided by Prof. Yonggang Liu (Beijing University of Chinese

Table 1. The growth inhibition rates (%) of human gastric cancer SGC-7901 cells treated with beta-Carboline alkaloids

beta-Carboline alkaloids (μg/ml)	24 hours	48 hours
0	0.00 ± 21.23%	0.00 ± 23.14%
10	25.65 ± 3.98%**	20.97 ± 14.68%**
20	35.05 ± 7.48%**	35.90 ± 10.73%**
40	88.19 ± 0.91%**	94.43 ± 1.57%**

Note: SGC-7901 cells were treated with beta-Carboline alkaloids (0, 10, 20 and 40 μg/mL) for 24 or 48 hours. The cellular viability was detected using Cell Counting Kit-8. The cell growth inhibition rate (%) was calculated using the formula: (1-experimental group optical density value/control group optical density value) × 100. One-Way ANOVA, $F = 60.933$, compared with 0 μg/ml beta-Carboline alkaloids, $^{**}P < 0.01$.

Medicine). Cell Counting Kit-8, Apoptosis DNA Ladder Extraction Kit and Hoechst Staining Kit were purchased from Beyotime Institute of Biotechnology (Haimen, Jiangsu, China). TRIzol® Reagent was purchased from Invitrogen. The agarose was purchased from Sangon Biotech Co. Ltd. (Shanghai, China). BCA Protein Assay Kit was purchased from Tiangen Biotech Co., Ltd. (Beijing, China). SuperSignal West Pico Chemiluminescent Substrate, the Prestained Protein Molecular Weight Marker (cat#SM0671) and Peroxidase-conjugated Affinipure Rabbit Anti-Goat IgG (H+L) were purchased from Thermo Scientific. Anti-β-actin polyclonal antibody (cat#ab8227), anti-PTEN monoclonal antibody (cat#ab32199) and anti-ERK1+ERK2 polyclonal antibody (cat#ab17942) were purchased from Abcam. Human gastric cancer SGC-7901 cells were purchased from Shanghai Institutes for Biological Sciences, Chinese Academy of Cell Resource Center. The cells were cultured in RPMI-1640 (Life Technologies) supplemented with 10% fetal bovine serum (Life Technologies) and placed in an atmosphere of 95% air/5% CO_2 at 37°C.

Cell counting Kit-8 (CCK-8) assay

SGC-7901 cells were seeded in 96-well plates (5×10^4 cells/ml, 200 μl/well) during the logarithmic growth phase. Twenty-four hours after seeding, the cells were treated with 200 μl of β-carboline alkaloids (0, 10, 20, and 40 μg/ml) for 24 and 48 hours at 37°C. The cellular viability was detected using the CCK-8 kit according to the manufacture's instruments. The cell growth inhibition rate (%) was calculated using the formula: (1 - experimental group optical density value/control group optical density value) × 100.

Hoechst 33258 staining

SGC-7901 cells were seeded at a concentration of 1×10^6 cells per 25 cm^2 culture flask during the logarithmic growth phase. Twenty-four hours after the seeding, the cells were treated with β-carboline alkaloids (0, 10, 20 and 40 μg/mL) for 48 hours at 37°C. Then the cells were treated with 0.25% trypsin, washed with PBS and dropped on a slide. After fixation with methanol/glacial acetic acid (3:1) for 10 min, the cells were stained with Hochest33258 (5 mg/l) for 45 min in the dark, observed under upright fluorescence microscope and photo graphed.

DNA fragmentation analysis

SGC-7901 cells were treated with β-carboline alkaloids (0, 10, 20 30 and 40 μg/ml) for 48 hours. The 5-FU (40 μg/ml) was used as the positive control. Fragmented DNA was isolated by the Apoptosis DNA Ladder Extraction Kit according to the manufacturer's instructions. The purified DNA was electrophoresed on a 1.2% agarose gel at 100 V for 25 min. DNA bands were visualized with UV light and photographed.

Quantitative real-time PCR (qRT-PCR) assay

SGC-7901 cells were treated with β-carboline alkaloids (0, 10, 20, 30 and 40 μg/ml) for 48 hours. The 5-FU (40 μg/ml) was used as the positive control. Then the cells were harvested and total RNA was prepared using the Trizol Reagent. Total RNA (5 μg) in a 20 μL reaction volume was reversely transcribed to form cDNA using the M-MLV First Strand Synthesis kit (Invitrogen; C28025-032). qRT-PCR analysis of mRNA levels were performed using the ABI7500 Real-Time PCR System (ABI, USA) with SYBR Select Master Mix (ABI, USA). The following primers were used for the PCR reactions: PTEN, 5'-TTTGAAGACCATAACCCACC-3' and 5'-ATTACACCAGTTCGTCCCTT-3'; ERK, 5'-TCACACAGGGTTCCTGACAG-3' and 5'-ATGCAGCCTACAGACCAAAT-3', β-actin, 5'-TGACGTGGACATCCGCAAAG-3' and 5'-CTGGAAGGTGGACAGCGAGG-3'. PCR procedures were template denaturation at 95°C for 15 sec and 40 cycles of 60°C for 1 min. The $2^{-\Delta\Delta CT}$ method was used to calculate

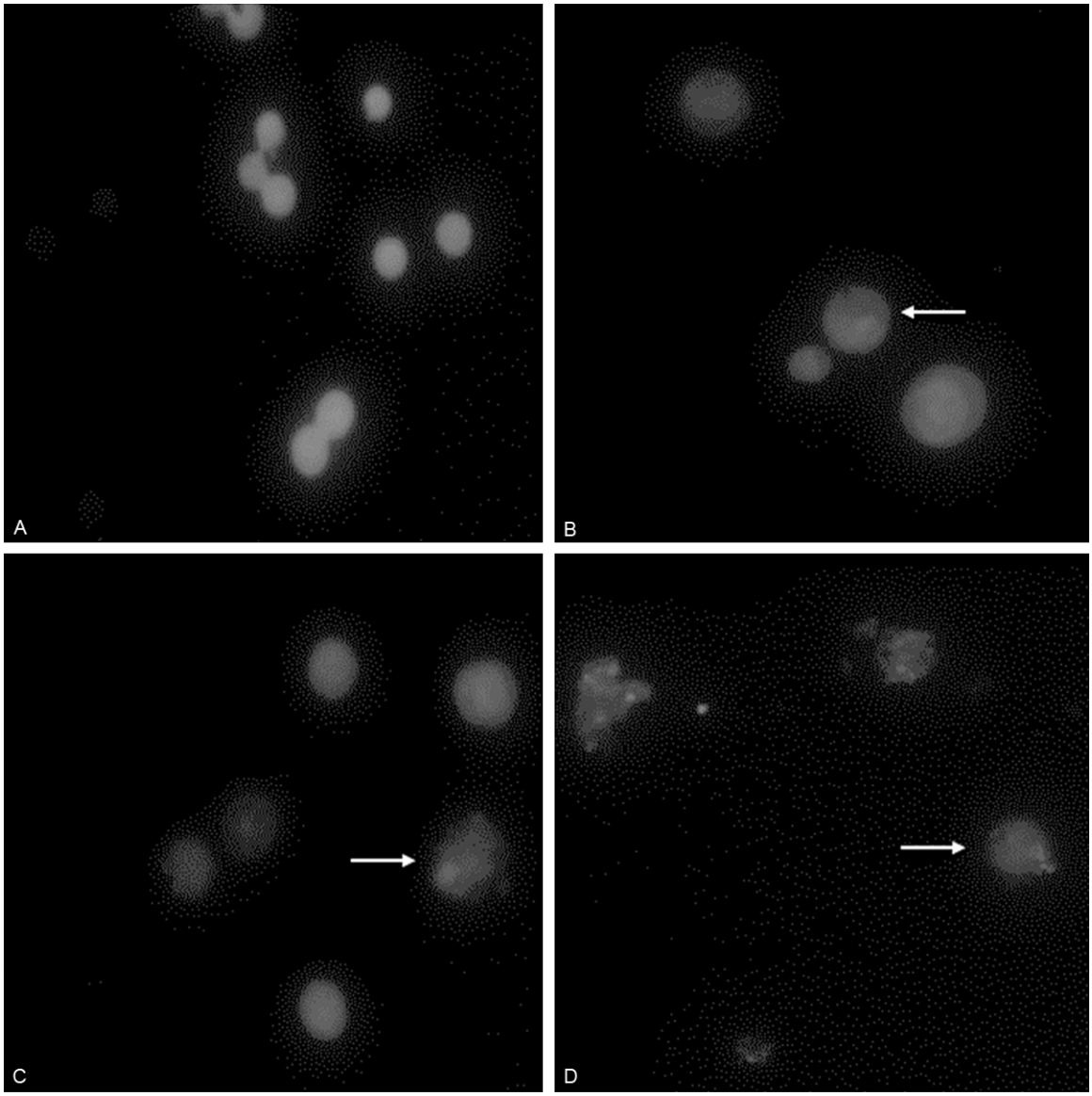

Figure 1. Apoptotic morphology induced by β-carboline alkaloids in SGC-7901 cells (magnification × 400). SGC-7901 cells were treated with β-carboline alkaloids at the concentration of 0 μg/ml (A), 10 μg/ml (B), 20 μg/ml (C) and 40 μg/ml (D) for 48 hours and the representative results were shown. The arrowhead indicates the apoptotic nuclei.

the expression of PTEN and ERK genes relative to the endogenous control gene β-actin.

Western blot analysis

SGC-7901 cells were treated with β-carboline alkaloids (0, 10, 20, 30 and 40 μg/mL) for 48 hours. The 5-FU (40 μg/mL) was used as the positive control. Then the cells were harvested. The concentrations of the total proteins were determined using BCA Protein Assay Kit. Twenty μg of total proteins were separated on 10% SDS/PAGE gels, and then transferred to nitrocellulose membranes for western blot analysis. After blocking with 5% non-fat milk for 1 hour, the membranes were incubated overnight with anti-PTEN antibody or anti-ERK1+ERK2 antibody. After washing, the membrane was then incubated with goat anti-rabbit HRP-conjugated IgG. Bound antibodies were detected using SuperSignal West Pico Chemiluminescent Substrate. The luminescence imaging was obtained using ChemiScope 3000. β-actin was used as an internal control.

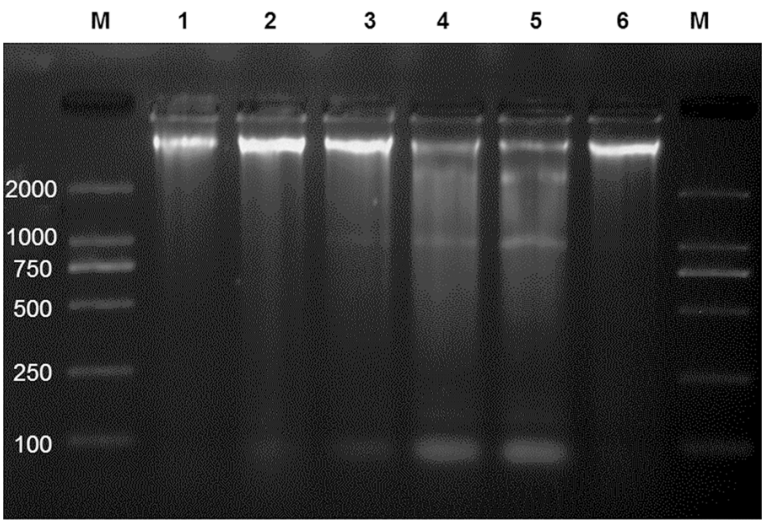

Figure 2. DNA ladder indicative of apoptosis induced by β-carboline alkaloids in SGC-7901 cells. SGC-7901 cells were treated with β-carboline alkaloids (0, 10, 20, 30 and 40 μg/mL) for 48 hours and DNA fragmentation analysis was performed. The 5-Fluorouracil (5-FU) (40 μg/mL) was used as the positive control. M, DNA marker (D2000); 1, 0 μg/mL β-carboline alkaloids; 2, 10 μg/mL β-carboline alkaloids; 3, 20 μg/mL β-carboline alkaloids; 4, 30 μg/mL β-carboline alkaloids; 5, 40 μg/mL β-carboline alkaloids; 6, 40 μg/mL 5-FU.

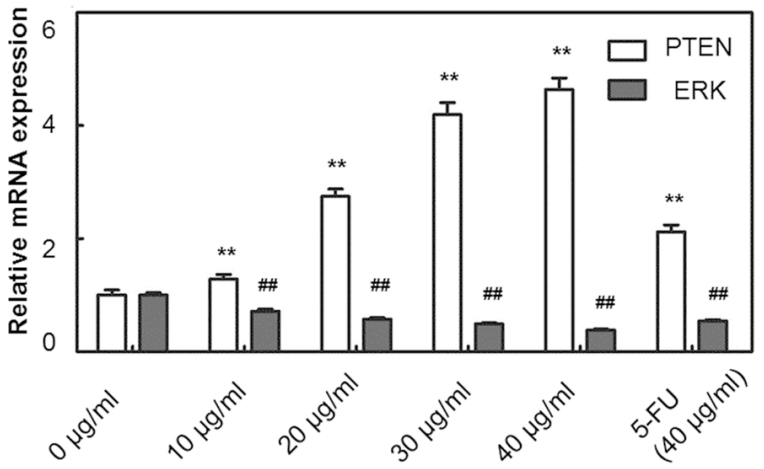

Figure 3. β-carboline alkaloids increase PTEN and decrease ERK mRNA expression in SGC-7901 cells. SGC-7901 cells were treated with β-carboline alkaloids at indicated concentrations for 48 hours. The 5-FU (40 μg/mL) was used as the positive control. PTEN and ERK mRNA levels were measured by qRT-PCR. The $2^{-\Delta\Delta CT}$ method was used to calculate the expression of PTEN and ERK genes relative to the endogenous control gene β-actin. The data represents mean ± SD of three independent experiments. One-Way ANOVA, $^{**}P < 0.01$ versus PTEN expression of the control group (0 μg/mL) and $^{\#\#}P < 0.01$ versus ERK expression of the control group (0 μg/mL).

Statistical analysis

All data were processed using SPSS17.0 statistical package. The data were presented as means ± standard deviation. One-Way ANOVA and post hoc test was performed to determine statistical significance of the differences. *P*-values of less than 0.05 were considered statistically significant.

Results

β-carboline alkaloids inhibit the growth of human gastric cancer SGC-7901 cells

To investigate the effects of β-carboline alkaloids on the growth of human gastric cancer SGC-7901 cells, CCK-8 assay was performed. SGC-7901 cells were treated with β-carboline alkaloids at 10, 20 and 40 μg/mL for 24 hours. The growth inhibition rates against the control (0 μg/mL) were 25.65 ± 3.98%, 35.05 ± 7.48% and 88.19 ± 0.91%, respectively, which were all significantly higher than that in the control group (0.00 ± 21.23%, $P < 0.01$). SGC-7901 cells were treated with β-carboline alkaloids at 10, 20 and 40 μg/mL for 48 hours, the growth inhibition rates against the control were 20.97 ± 14.68%, 35.90 ± 10.73% and 94.43 ± 1.57%, respectively, which were all significantly higher than that in the control group (0.00 ± 23.14%, $P < 0.01$) (**Table 1**). These results indicate that β-carboline alkaloids inhibit the growth of SGC-7901 cells concentration dependently.

β-carboline alkaloids increase the apoptosis of SGC-7901 cells

To determine the effects of β-carboline alkaloids on apoptosis of SGC-7901 cells, Hoechst 33258 staining and DNA fragmentation analysis were performed. Hoechst 33258 staining was used to visualize nuclear changes and apoptotic mor-

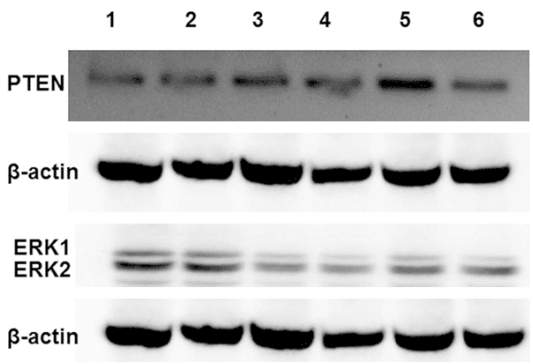

Figure 4. β-carboline alkaloids increase PTEN and decrease ERK protein expression in SGC-7901 cells. SGC-7901 cells were treated with β-carboline alkaloids (0, 10, 20, 30 and 40 μg/mL) for 48 hours. The 5-FU (40 μg/mL) was used as the positive control. PTEN and ERK protein expression was detected using western blot analysis. β-actin was used as an internal control. Representative results were shown. 1, 0 μg/mL β-carboline alkaloids; 2, 10 μg/mL β-carboline alkaloids; 3, 20 μg/mL β-carboline alkaloids; 4, 30 μg/mL β-carboline alkaloids; 5, 40 μg/mL β-carboline alkaloids; 6, 40 μg/mL 5-FU.

phology including nuclear condensation, chromatin condensation, nuclear fragmentation and formation of apoptotic bodies. The results shown in **Figure 1** revealed the appearance of apoptotic nuclei upon β-carboline alkaloids treatment at indicated concentrations for 48 hr.

To further confirm the results of Hoechst 33258 staining, GC-7901 cells were treated with β-carboline alkaloids (0, 10, 20, 30 and 40 μg/mL) for 48 hours and DNA fragmentation analysis was performed. As shown in **Figure 2**, β-carboline alkaloids induced DNA ladder, indicative of apoptosis in SGC-7901 cells concentration-dependently. These results indicate that β-carboline alkaloids increase the apoptosis of SGC-7901 cells.

β-carboline alkaloids increase PTEN and decrease ERK mRNA expression in SGC-7901 cells

To study the effects of β-carboline alkaloids on PTEN and ERK mRNA expression in SGC-7901 cells, qRT-PCR assay was performed (**Figure 3**). SGC-7901 cells were treated with β-carboline alkaloids at indicated concentrations for 48 hours. The cells treated with 0 μg/mL β-carboline alkaloids were used as the negative control. As the concentration of β-carboline alkaloids increased from 10, 20, 30, to 40 μg/mL, PTEN mRNA expression increased from 1.280 ± 0.152, 2.749 ± 0.226, 4.193 ± 0.375, to 4.637 ± 0.357, which were all significantly higher than that of the negative control (1.007 ± 0.146, $P < 0.01$). As the concentration of β-carboline alkaloids increased from 10, 20, 30, to 40 μg/mL, ERK mRNA expression decreased from 0.719 ± 0.055, 0.577 ± 0.045, 0.494 ± 0.029, to 0.384 ± 0.021, which were all significantly lower than that of the negative control (1.002 ± 0.068, $P < 0.01$). These results indicate that β-carboline alkaloids increase PTEN and decrease ERK mRNA expression in SGC-7901 cells in a concentration dependent manner.

β-carboline alkaloids increase PTEN and decrease ERK protein expression in SGC-7901 cells

To confirm the results of PTEN and ERK mRNA expression mentioned above, the levels of PTEN and ERK protein were detected using western blot analysis (**Figure 4**). As the concentration of β-carboline alkaloids increased from 0 to 40 μg/mL, PTEN protein expression increased gradually with the peak at 40 μg/mL and ERK protein expression decreased gradually with the peak at 30 μg/mL. These results indicate that β-carboline alkaloids increase PTEN and decrease ERK protein expression.

Discussion

In recent years, the incidence of gastric cancer increased year by year in China and radical surgery is the only means of achieving a cure. The patients with stage II-IV gastric cancer are at high risk of recurrence and distant metastasis [13]. In recent years, anti-tumor mechanism of Traditional Chinese Medicine has aroused much attention. It has been reported that Harmine and its derivatives could induce apoptosis in gastric cancer cells, pancreatic cancer cells, leukemia cells and HepG2 cells [3, 14-16]. In this study, the cytotoxic effects of β-carboline alkaloids on human gastric cancer SGC-7901 cells were investigated. The β-carboline alkaloids used in this study were purified from the alkaloids of Peganum harmala using electrospray ionization mass spectrometer to avoid the interference of other components in Peganum harmala.

β-carboline alkaloids inhibit the growth and induce apoptosis of SGC-7901 cells. Treatment of SGC-7901 cells with β-carboline alkaloids at

different concentrations for 48 hours resulted in increased PTEN and decrease ERK expression. It seems that PTEN and ERK participated in β-carboline alkaloids induced apoptosis of SGC-7901 cells. This might be because β-carboline alkaloids could break the balance between PTEN and ERK, inhibit the Ras/Raf/MEK/ERK signal pathway and induce apoptosis in the cells.

It has been reported that inhibition of ERK pathway sensitizes gastric cancer cells to chemotherapeutic drugs [17]. In this study, β-carboline alkaloids decreased ERK expression in gastric cancer SGC-7901 cells. Whether β-carboline alkaloids could also sensitize gastric cancer cells to chemotherapeutic drugs deserves further investigation.

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Disclosure of conflict of interest

None.

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