ELSEVIER

Archives of Medical Research ■ (2018) ■

Archives of Medical Research

ORIGINAL ARTICLE

Inhibitory Effect of Artesunate on Growth and Apoptosis of Gastric Cancer Cells

Lei Wang,[a] Liang Liu,[b] Jing Wang,[b] and YueTong Chen[b]

[a]*Department of Thoracic Surgery,* [b]*Tumor Institute, The Fourth Hospital of Hebei Medical University, Shijiazhuang, Hebei, P.R. China*

Received for publication August 15, 2017; accepted March 2, 2018

Objective. The present study aimed to explore the inhibitory effect of artesunate on the growth and apoptosis of gastric cancer cells, in order to search for high effective and low toxic anti-gastric cancer drugs.

Methods. Flow cytometry was used to detect the CDC25A protein expression in different gastric diseases. After the treatment of different concentrations of artesunate (0, 30, 60, 120 μmol/l) on the gastric cancer cell line SGC-7901 cells for 24 h, 0 μmol/l Art was instead of normal saline as control group, the expression of CDC25A, Bcl-2, Bax, Caspase-3 protein, mitochondrial membrane potential, cell apoptosis and cell cycle were detected by flow cytometry.

Results. The CDC25A protein expression in gastric adenocarcinoma was significantly higher than that in normal gastric tissues. After the treatment of different concentrations of Art, the apoptosis rate of SGC-7901 cells in Art groups was significantly higher than that in control group. The proliferation index and CDC25A protein of SGC-7901 cells in Art groups was significantly lower than that in control group. Compared with control group, the Bcl-2 protein and mitochondrial membrane potential was significantly lower but the Bax and Caspase-3 protein expression level was significantly higher.

Conclusions. The high expression of CDC25A protein in gastric adenocarcinoma is involved in the occurrence and development of gastric adenocarcinoma. Artesunate can inhibit the growth of gastric adenocarcinoma cells SGC-7901 and induce the cell apoptosis, the mechanism may be related to the regulation of CDC25A, Bcl-2, Bax, Caspase-3 and mitochondrial membrane potential in SGC-7901 cells. © 2018 IMSS. Published by Elsevier Inc.

Key Words: Gastric cancer, Artesunate, Proliferation, Inhibitory effect.

Introduction

Gastric cancer is one of the most common malignant tumors in the world, with high morbidity and mortality (1−3), which is a serious threat to people's life and health. At present, chemotherapy is one of the conventional treatment methods of gastric cancer. However, because of the high side effects and drug resistance of chemotherapy drugs, it often affects the efficacy of chemotherapy and even leads to the failure of chemotherapy. So it is very important to look for anticancer drugs with high efficiency and low toxicity. Traditional Chinese medicine has a long history in the treatment of tumors with the property of low toxicity and low cost. Art (artesunate) is a derivative of artemisinin, mainly from the plant artemisia annua. Art is a highly effective and low toxic antimalarial drug (4).Recent studies have found that Art also has antitumor activity (5−7). A large number of studies have reported that Art can inhibit the growth and induce apoptosis of several tumor cells, but few studies have been done in gastric cancer cells, and the specific mechanisms have not been elucidated. In our previous study, we found that Art can inhibit the growth of esophageal cancer cells by inducing cell cycle

Conflict of interest: The authors declare that they have no conflict of interest.

Address reprint requests to: Liang Liu, Tumor Institute, The Fourth Hospital of Hebei Medical University, Shijiazhuang, Hebei 050011, P.R. China; Phone: (+86) 18531115751; FAX: (+86) 0311-86077634; E-mail: aliangdaziran@163.com

0188-4409/$ - see front matter.
https://doi.org/10.1016/j.arcmed.2018.03.004

arrest and apoptosis of esophageal cancer cells (8). This paper studied the relationship between the occurrence and development of gastric cancer and the expression of CDC25A and inhibited effect of Art on the growth and apoptosis of gastric cancer cell line SGC-7901. In our paper, we also researched the levels of CDC25A, Bcl-2, Bax, Caspase-3 and mitochondrial membrane potential in SGC-7901 cells regulated by Art, so as to investigate the mechanism of Art inhibiting SGC-7901 cell growth and inducing apoptosis, in order to provide high efficiency and low toxicity of anti-cancer drugs for clinic.

Materials and Methods

Sample collection. Clinical samples (gastric carcinoma tissue) were collected from the Fourth Hospital of Hebei Medical University, Hebei, China. 40 samples from gastric carcinoma tissues were collected with complete information from June 2014−June 2015. In order to obtain the 40 normal (control) samples, 0.5 cm × 0.5 cm sized gastric tissues were randomly collected at the time of routine sampling, from regions located >5 cm away from the cancer tissues. If these samples were confirmed to be normal tissues through pathological examination, they were used in the study as samples from normal gastric tissues (the control group). This study was approved by the Ethic Committee of the Fourth hospital of Hebei Medical University.

Chemicals and reagents. Artesunate was purchased from Guilin Pharmaceutical Co. (Guangxi, China). FITC labeled CDC25A antibodies were purchased from the SANTA CRUZ Co. (SANTA CRUZ, USA). AnnexinV-FITC/PI kit was purchased from the Beckman Coulter Co. (Beckman Coulter, Miami, FL, USA). Propidium iodide was purchased from the BD Co. (BD, USA).

Cells and cell culture. The human gastric cancer cell line SGC-7901 was obtained from the Cell repository of Chinese Academy of Sciences (Shanghai, China). Cells were cultured in RPMI 1640 medium (Gibco-BRL, Life Technologies, Paisley, Auckland) supplemented with 10% fetal bovine serum (Gibco-BRL, Life Technologies, Paisley, Auckland), 100 units/mL penicillin (North China Pharmaceutical Co., Ltd., Shijiazhuang, China) and 100 μg/mL streptomycin (North China Pharmaceutical Co., Ltd., Shijiazhuang, China) at 37°C in a humidifed atmosphere of 5% CO2.

Detection of CDC25A protein expression in samples using flow cytometry. The samples from cancer tissues and normal tissues were washed twice with cold PBS. The tissues were then made into single cell suspensions by the net-twist method. The cell concentration was adjusted to 1×10^7/mL. For each sample, 100 μl of cell suspension was dispensed into the test tube, and 10 μl of FITC-CDC25A antibody was added into it. The cells were stained in a dark place for 30 min, and then washed once with cold PBS. Afterwards, 1 mL of PBS was added into the cells, and the CDC25A protein of the cells was assessed using an FC500 flow cytometer (Beckman Coulter, Fullerton, CA, USA). The expression of CDC25A protein was expressed by mean fluorescence intensity.

Cell experiment groups and drug intervention experiments. SGC-7901 cells in the logarithmic phase were collected and cultured in culture flask at the density of 5×10^6. After the cells reached 80-85% confluence, artesunate was added into each culture flask, at the concentration of 30, 60, 120 μmol/l, normal saline instead of artesunate as control group. After the artesunate treatment for 24 hours, SGC-7901 cells was collected. The cell concentration was adjusted to 1×10^6/ml. Each experiment was repeated 3 times. SGC-7901 cells treated by NS and 30, 60, 120 μmol/l Art were named NS and 30, 60, 120 μmol/l Art group respectively.

Assessing cell apoptosis using flow cytometry. 1 mL of the single cell suspension (containing 1×10^6 cells) was prepared by the above (as described in the section 'Cell experiment groups and drug intervention experiments'). 1 mL of cell suspension was collected, washed with cold PBS once, and suspended in 100 μl of 1 × binding buffer. Afterwards, 10 μl of Annexin V-FITC was added, and the mixture was placed on ice for 15 min in the dark. Next, 380 μl of 1 x binding buffer and 10 μl of PI were added, incubated on ice for 15 min in the dark, washed with cold PBS once, and suspended with 1 mL of PBS. The apoptosis of the cells was measured using flow cytometry. The EXPO32 ADC software (Beckman Coulter) was used to analyze the immunofluorescence data and evaluate the apoptosis rate.

Assessing cell cycle distribution using flow cytometry. 1 mL of the single cell suspension (containing 1×10^6 cells) was prepared by the above (as described in the section 'Cell experiment groups and drug intervention experiments'), washed with cold PBS twice, and fixed with 70% ethanol at 4°C for 24 h. Then, the cells were again washed with PBS twice, and 1 mL of propidium iodide was added. After incubating at 4°C for 30 min, flow cytometry was performed and MultiCycle AV software (Beckman Coulter) was used to analyze the cell cycle. The proliferation status was expressed by proliferation index.

$$\text{Proliferation } \textit{Index } (PI) = (S + \mathrm{G_2/M})/(\mathrm{G_0/G_1} + S + \mathrm{G_2}/M) \times 100\%$$

Assessing the expression of CDC25A protein using flow cytometry. 1 mL of the single cell suspension (containing 1×10^6 cells) was prepared by the above (as described in the section 'Cell experiment groups and drug intervention experiments'). 1 mL of cell suspension was collected, washed with cold PBS once and suspended with 100 μl of PBS, 10 μl of FITC-CDC25A antibody was added, incubated for 30 min in the dark, washed with cold PBS once, and suspended with 1 mL of PBS. The CDC25A protein in