JTCM 中医杂志 Journal of Traditional Chinese Medicine

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J Tradit Chin Med 2017 April 15; 37(2): 229-235
ISSN 0255-2922

EXPERIMENTAL STUDY

Baicalin extracted from Huangqin (*Radix Scutellariae Baicalensis*) induces apoptosis in gastric cancer cells by regulating B cell lymphoma (Bcl-2)/Bcl-2-associated X protein and activating caspase-3 and caspase-9

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Supported by the Natural Science Foundation of Gansu Province (No. 148RJZA073); the Health Industry Scientific Research Project of Gansu Province (No. GWGL 2013-40); and the Openning Plan Project of Key Laboratory for Transfer of Dunhuang Medicine at the Provincial and Ministerial Level (No. DHYX1213-008)
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Accepted: June 1, 2016

Abstract

OBJECTIVE: To evaluate the effects of baicalin in human gastric cancer cells, including apoptosis-inducing effects, and to investigate its underlying mechanisms of action.

METHODS: Cell proliferation and apoptosis assays were performed to investigate the anti-proliferation effects of baicalin in human gastric cancer BGC-823 and MGC-803 cells. Real time-quantitative polymerase chain reaction and Western blotting analysis were performed to elucidate the molecular mechanisms underlying the anti-tumor properties of baicalin.

RESULTS: In BGC-823 and MGC-803 gastric cancer cells treated with 80, 120, and 160 μmol/L baicalin for 48 h, a 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide assay showed that baicalin significantly inhibited cell proliferation in a dose-dependent manner, while flow cytometric analysis demonstrated that baicalin could induce apoptosis, also in a dose-dependent manner. Moreover, baicalin up-regulated the expression of caspase-3, caspase-9, and B cell lymphoma (Bcl-2)-associated X protein and down-regulated the expression of Bcl-2 at both the mRNA and protein level.

CONCLUSION: Baicalin has potential as a therapeutic agent for gastric cancer by inducing apoptosis in cancer cells.

Keywords: Baicalin; Stomach neoplasms; ApoptosisLymphoma, B-Cell; Bcl-2-associated X protein; Caspases, effector

INTRODUCTION

Epidemiology data show that gastric cancer is the fifth most common cancer and the second most common

cause of cancer-related mortality globally,[1] Despite recent advances in surgical techniques and the development of adjuvant therapy forgastric cancer, treatment strategies remain insufficient. Chemotherapy is an important therapeutic approach, but most chemotherapeutic agents used to treatgastric cancer have serious side effects such as hematologic toxicity, gastrointestinal disorder, and alopecia. Therefore, the intolerable toxicity of chemotherapy has limited its clinical application. In recent years, research has focused on the application of natural pharmacological agents for treating cancer cells. Many natural products, including Chinese herbs, have been shown to exert significant anti-tumor effects by blocking the cell cycle, inducing cancer cell apoptosis, or regulating other characteristics of cancers. The clinical application of natural products such as flavonoids and alkaloids for treating cancers has significant advantages, such as fewer side effects, increased safety, and better tolerability.

Baicalin is a natural flavonoid extracted from Huangqin (*Radix Scutellariae Baicalensis*). It has been shown to exert anti-tumor effects in several types of cancer, *in vivo* and *in vitro*, including hepatocellular carcinoma,[1,2] hepatoblastoma cancer,[3] lung cancer,[4] pancreatic cancer,[5] colorectal cancer,[6] mucoepidermoid carcinoma,[7] breast cancer,[8-10] bladder cancer,[11,12] prostate cancer,[13,14] myeloid leukemia,[15-17] Burkitt lymphoma,[18] and multiple myeloma.[19] The anti-cancer properties of baicalin are associated with its ability to induce cell cycle arrest[2,20] and apoptosis,[1-18] reverse tumor multidrug resistance,[16] suppress invasion and metastasis,[21] inhibit epithelial-mesenchymal transition,[22] and trigger autophagy.[1,11] However, apoptosis induced by baicalin in gastric cancer cells has not been reported to date. Therefore, the present study was designed to assess the effects of baicalin on human gastric cancer BGC-823 and MGC-803 cell lines and to determine whether baicalin may induce gastric cancer cell apoptosis and explore the mechanisms involved in this process.

MATERIALS AND METHODS

Cell lines and cell culture

BGC-823 and MGC-803 human gastric cancer cell lines were obtained from The Tumor Institute and Hospital, Chinese Academy of Medical Sciences, Beijing, China. Cells were grown in 90% Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μmol/L streptomycin, and incubated at 37 ℃ in a humidified atmosphere of 5% CO_2.

Baicalin treatment

Baicalin (Baoji Fangsheng Pharmaceutical Co., Ltd., Xi'an, China) was analyzed by high-performance liquid chromatography (HPLC), dissolved in dimethyl sulfoxide (DMSO) (Sigma, St. Louis, MO, USA) to a final DMSO concentration less than 0.5%, and diluted in DMEM. BGC-823 and MGC-803 human gastric cancer cell cultures were treated with baicalin for 24, 48, 72, and 96 h at 40, 80, 120, and 160 μmol/L to detect cell proliferation.

Cell proliferation assay

After treatment with various concentrations of baicalin, cells were trypsinized, counted, plated in triplicate in 96-well plates (3000 cells /well), and incubated at 37 ℃ for 24, 48, 72, and 96 h. Next, 20 μL of 5 μmol/L3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide (MTT) (Sigma-Aldrich, St. Louis, MO, USA) was added in phosphate buffered saline (PBS) was added to each well, and the plate was incubated at 37 ℃ for 4 h. The plate was then centrifuged at 1000 × *g* for 2 min and medium was removed. DMSO (150 μL) was then added per well. After incubation at 37 ℃ for 5 min, absorbance was measured spectrophotometrically at 570 nm using a Benchmark microtiter plate reader (Bio-Rad Laboratories, Hercules, CA, USA).

Apoptosis assay

Following baicalin treatment at concentrations of 80, 120, and 160 μmol/L for 48 h, cells were trypsinized and washed with phosphate buffered saline (PBS) and resuspended in 100 μL of binding buffer. Annexin V (BD Biosciences, New York, NY, USA) staining was performed according to the manufacturer's instructions. In brief, 5 μL annexin V-Fluorescein isothiocyanate (FITC) (2 μmol/L) and 5 μL Propidium Iodide (PI) (2 μmol/L) were added to cells for 15 min in dark conditions. The percentage of apoptotic cells was determined by flow cytometry (BD Biosciences, New York City, NY, USA).

RT-qPCR analysis

Total RNA was extracted from cell lines using Trizol reagent (invitrogen, Life Technologies, Carlsbad, USA). cDNA was synthesized using AccuPower® RT PreMix (Bioneer, Shanghai, China). Reverse transcription was performed as follows: the transcription mixture was incubated at 70 ℃ for 5 min and then placed on ice; cDNA synthesis reaction was performed for 60 min at 42 ℃ and 5 min at 94 ℃, and then held at 4 ℃. Synthesized cDNA products were diluted 3- to 5-fold. Real time-quantitative polymerase chain reaction (RT-qPCR) was performed in triplicate using an ABI7500 real-time PCR system (ABI, Carlsbad, CA, USA) using 1 μL diluted cDNA as the template in a 20 μL reaction volume with AccuPower® Green Star qPCR MasterMix (Bioengineer, Shanghai, China). The PCR reaction was carried out as follows: denaturing at 95 ℃ for 10 min followed by 40 cycles at 94 ℃ for 30 s and 60 ℃ for 30 s. Next, melting curve analyses were performed to validate the specificity of the expected PCR product. The primers used for RT-qPCR were purchased from TaKaRa Co., Ltd., (Dalian, China) (Table 1). Relative expression was calculated using the $2^{-\Delta\Delta CT}$ method.