BAICALIN INHIBITS THE PROLIFERATION OF GASTRIC CANCER CELLS AND INDUCES APOPTOSIS THROUGH THE DEATH RECEPTOR PATHWAY

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ABSTRACT

Objective: To analyze the mechanism of baicalin inhibiting the proliferation of human gastric cancer cells and inducing apoptosis through death receptor pathway.

Methods: The human gastric cancer cell line (BGG-823) was cultured in vitro and was randomly divided into control and baicalin groups with 4 concentrations (20, 40, 80, 160 μmol/L). The proliferation of gastric cancer cells after treatment with different concentrations of baicalin was detected using an MTT method. The apoptosis of cells was detected by flow cytometry. The expression of caspase3, caspas8, FASL, FAS and TRAIL gene and protein were measured by RT-PCR and western blot assay.

Results: The results of the MTT assay showed that the proliferation of the BGC-823 cell line could be significantly inhibited at time points of 24, 48, 72 and 96 h, and the proliferation rate decreased clearly with the increase of drug concentration (P<0.05). The results of flow cytometry showed that baicalin could significantly increase the apoptosis rate of cells, and the apoptosis rate gradually increased with the increase of the drug concentration (P<0.05). The results of RT-PCR and western blot assay indicated that the expression of caspase3, caspas8, FASL, FAS and TRAIL mRNA and protein in gastric cancer cells increased gradually with the increase of drug concentration (P<0.05).

Conclusion: Baicalin can significantly inhibit the proliferation and induce apoptosis of human gastric cancer BGG-823 cells, and its related factors may be related to the death receptor pathway.

Keywords: Baicalin, death receptor pathway, gastric cancer cells, proliferation, apoptosis, relevant mechanism.

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Introduction

Gastric cancer is the second most common cancer in the world. It ranks first in the incidence of all kinds of malignant tumours in China(1). Many studies have suggested that it is related to environmental, dietary factors, helicobacter pylori (HP) infection, heredity and so on, all of which frequently occur in middle-aged and elderly people over 50 years old. At present, surgery is still the first choice for the treatment of gastric cancer. Because most patients have no obvious or typical symptoms in the early stage, they often reach the local late stage at the time of diagnosis and have lost the opportunity for surgical treatment. Hence, chemotherapy is still the auxiliary treatment method before and after surgery; however, adverse reactions increase, patient tolerance is poor and the 5-year survival rate is approximately 30%(2,3).

In recent years, the level of apoptosis-related molecules has been found to be closely related to the occurrence and development of gastric cancer(4). Apoptosis involves a process of cell death, in which the death receptor pathway is more extensively activated than the signal transduction pathway. The known death receptors are tumour necrosis factor receptor (TNFR), factors associated suicide (FAS) and TNF-related apoptosis-inducing ligand (TRAIL). Each pathway in which each receptor is located mainly transmits extracellular signals to the cell through transmembrane receptors, and the cascade reaction between binding proteins and executive proteins leads to apoptosis(5).
Baicalin is a flavonoid compound. Modern pharmacological studies have shown that baicalin leads to clearing away of heat and detoxification, resisting pathogens, protecting against liver injury, repairing brain injury and has strong physiological effects such as anti-cancer reactions. However, there are few reports regarding baicalin on the proliferation and apoptosis of human gastric cancer cells. Therefore, by observing the effect of baicalin on apoptosis induced by the gastric cancer cell proliferation inhibitor, the role of the death receptor pathway in the inhibition of gastric cancer cell proliferation by baicalin is preliminarily discussed, an approach that provides a new idea for the treatment of gastric cancer.

Materials and methods

Materials

A human gastric cancer cell line (BGG-823) was purchased from the cell bank of the Chinese Academy of Sciences, Shanghai. Baicalin (purity>98%) was obtained from Baoji Fangsheng Biological Development Co., Ltd. Foetal calf serum was purchased from Zhejiang Tianhang Biotechnology Co., Ltd. The DMEM culture medium was obtained from Hangzhou Keyi Biology Co., Ltd. The cell proliferation and activity detection kit (CCK-8) was purchased from DOJINDO company (Japan). Rabbit anti-human FASL, TRAIL, caspase3, caspase8 and GAPDH monoclonal antibody were acquired from the Affinity company (US). Filter paper, glycine and sodium laurel sulphate were purchased from Shanghai Sangon Bioengineering Co., Ltd.

An Ultra-clean workbench and CO₂ incubator were purchased from Shanghai Jinghong experimental equipment Co., Ltd. The low-temperature and high-speed centrifuge was purchased from Beckman company (US). -20 °C and -80 °C refrigerators were obtained from Qingdao Haier. An inverted optical microscope was purchased from the Olympus company (Japan). Developer and fixed liquid, enzyme labelling instrument, gel imager and western blot detection system were acquired from Bio-Rad (US).

Cell culture

BGG-823 cells were cultured in the medium containing 10% foetal bovine serum under the conditions of 5% CO₂ volume fraction and saturated humidity of 37 oC. The cell culture medium was replaced every 2 to 3 days, and cells in logarithmic growth phase were selected in the experiment.

The proliferation of cells was detected using an MTT assay: the BGC-823 cell line in the logarithmic growth phase was digested with digestive fluid, added to the culture medium, and the density of cell suspension was adjusted by counting plate. According to the experimental design, 100 μL of single-cell suspension were added to each well, and the number of cells per well was 1 × 10⁴ / mL. The cell culture bottle was gently shaken to mix the cells before vaccination. After the cells planted on the 96-well plate adhered to the wall, the BGC-823 cell line was treated with 20, 40, 80 and 160 μmol / L baicalin for 24, 48, 72 and 96 h, respectively. After each time point treatment, 20 mL of aseptic MTT were added to each well to avoid light. The cells were conventionally cultured in the conditions such that the CO₂ volume fraction was 5% and the saturated humidity was 37 oC for 4 h. After 4 h of cultivation, the culture medium was gently sucked out, the formazan crystal was dissolved by adding 150ml/well dimethyl sulfoxide, and the crystal was completely dissolved by shaking for about 10 min. The absorbance value is measured by an enzyme labelling instrument, and the OD value of each vacancy is read. The cell proliferation rate was calculated. Cell proliferation rate = (experimental group - control group) OD value / (control group - blank group) OD value.

The apoptosis was detected by flow cytometry: the human gastric cancer cell line BGC-823 in the logarithmic growth phase was digested with digestive fluid, added to the culture medium, and the density of cell suspension was adjusted by counting plate. According to the experimental design, 100 μL of single-cell suspension were added to each well, and the concentration of cells per well was 2.5 × 10⁴ / mL. The cell culture bottle was gently shaken to mix the cells before vaccination. The cells were cultured in an incubator with a CO₂ volume fraction of 5% and a saturated humidity of 37 oC. After the cells adhered to the wall, the BGC-823 cell line was treated with 20, 40, 80, 160 μmol / L baicalin for 24, 48, 72 and 96 h, respectively. The control group was an untreated group, which was placed again in an incubator with CO₂ volume fraction of 5% and saturated humidity of 37 oC for 48 h. After 48 h, the cells were collected in the centrifuge tube and labelled. 48 h later, the cells were collected and placed in a centrifugal tube and marked. The cells were digested with digestive fluid, and then 1 mL culture medium was added to stop digestion. PBS buffer was used to clean the remaining cells from each well of the 6-well plate. After centrifugation at 800 r/min for 5 min, the supernatant was discarded, and the apoptosis rate in each group was detected.
The expression of caspase3, caspase8, FASL, FAS and TRAIL protein was detected by western blot assay. Cells in logarithmic growth phase were added to the culture medium containing 5 ml of 10% foetal bovine serum. After 24 h of culture, baicalin solution was added, and the final concentration of baicalin was 40, 80 and 160 μmol / L for a certain period of time. The cells were scraped with a cell curette, and the protein content was determined using the BCA method. The expression of caspase3, caspase8, FASL, FAS and TRAIL mRNA was detected by an RT-PCR assay.

Statistical method
All statistical data were analyzed using the SPSS20.0 software package. The measured data were expressed as mean±standard deviation (X±s). Analysis of variance (ANOVA) was used for the comparison of baicalin among different concentration groups, and an LSD method was used for comparison between two groups. P<0.05 represents that the results were statistically significant.

Results

Effect of baicalin on proliferation of gastric cancer BGG-823 cells
After treatment of the BGC-823 cell line with 20, 40, 80 and 160 μmol/L baicalin, the results of the MTT assay showed that baicalin could significantly inhibit the proliferation of BGC-823 at 24, 48, 72 and 96 h, and the proliferation rate was remarkably decreased with the increase of drug concentration. There was a significant difference between the different concentration groups (P<0.05). The results are shown in Figure 1.

Effect of baicalin on apoptosis of gastric cancer BGG-823 cells
After treatment of the BGC-823 cell line with 20, 40, 80 and 160 μmol/L baicalin, the results of flow cytomotomy showed that baicalin could significantly increase the apoptosis rate of cells, and the apoptosis rate increased gradually with the increase of drug concentration. There was a significant difference between the different concentration groups (P<0.05). The results are shown in Figure 2.

**Table 1:** Effect of baicalin on caspase3, caspase8, FASL, FAS and TRAIL mRNA expression in gastric cancer BGG-823 cells.
Note: *represents compared with the control group, P<0.05.
Effect of baicalin on caspase3, caspase8, FASL, FAS and TRAIL protein expression in gastric cancer BGG-823 cells

After treating the BGC-823 cell line with 40, 80 and 160 μmol/L baicalin, the results of western blot assay showed that the expression of caspase3, caspase8, FASL, FAS and TRAIL protein in gastric cancer BGG-823 cells increased gradually with the increase of drug concentration (P<0.05); results are shown in Figure 3.

Some scholars have found that baicalin can induce apoptosis of lung cancer, colon cancer and other tumour cells and inhibit the proliferation of various cancer cells through surface death receptors\(^{(10)}\). It has been reported for the first time that baicalin can inhibit the formation of gallbladder cancer cells by affecting the cell cycle and inhibiting colony formation. Baicalin treatment can significantly inhibit the expression of NF-κB in gallbladder cancer cells and induce apoptosis by up-regulating Bax and down-regulating Bcl-2 expression\(^{(11)}\). The results of this study suggested that, after treatment of the human gastric cancer cell line BGC-823 with 20, 40, 80 and 160 μmol/L baicalin, baicalin could significantly inhibit the proliferation of BGC-823 at 24, 48, 72 and 96 h, and the proliferation rate was remarkably decreased with increasing drug concentration (P<0.05). The results of flow cytometry indicated that baicalin could significantly increase the cells' apoptosis rate, which increased gradually with the increase of drug concentration (P<0.05). These results suggest that baicalin can inhibit the growth of gastric cancer cells in a time- and dose-dependent manner.

The tumour is caused by malignant cell proliferation and uncontrolled apoptosis, in which the FAS-mediated apoptosis pathway plays an important role in immunomonitoring and maintaining intracorporeal balance\(^{(12)}\). FAS is an important death receptor on the surface of the cell, and once the binding of the ligand FASL occurs trimerization results, and death signal transduction can be activated by the FAS molecule, leading to DD activation of the cytoplasmic segment of the FAS molecule; the caspase-8 zymogen is then activated, the caspase-cascade reaction is started, DNA repair and replication are closed, and induction of the formation of apoptotic bodies further leads to cellular apoptosis\(^{(13)}\). When FAS and FASL expression or function loss or abnormality leads to the inactivation of the FAS system, its apoptotic effect is significantly inhibited, which may promote the formation of malignant tumours\(^{(14)}\). TRAIL is also a representative factor of death receptor pathways and has been found to cause proliferation or apoptosis of tumour cells by inducing signalling pathways such as PI3K/Akt\(^{(15)}\).

In this study, the results of RT-PCR and western blot showed that the expression of caspase3, caspase8, FASL, FAS and TRAIL mRNA and protein in gastric cancer cells increased gradually with the increase of drug concentration (P<0.05), suggesting that the effect of baicalin on human gastric
cancer cells may be realized by activating related factors of the death receptor pathway. In conclusion, baicalin can significantly inhibit the proliferation and induce apoptosis of human gastric cancer BGG-823 cells, and its related factors may be related to the death receptor pathway.

References

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