PO Box 2345, Beijing 100023, China
www.wjgnet.com
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RAPID COMMUNICATION

Inhibitory effect of Fuzheng Yiliuyin in combination with chemotherapeutics on human gastric carcinoma cell strain

Yi Liu, Rui Wang, Gen-Quan Qiu, Ke-Jun Nan, Xi-Cai Sun

Yi Liu, Rui Wang, Gen-Quan Qiu, Xi-Cai Sun, Department of TCM, First Hospital of Xi'an Jiaotong University, Xi'an 710061, Shaanxi Province, China
Ke-Jun Nan, Department of Oncology, First Hospital of Xi'an Jiaotong University, Xi'an 710061, Shaanxi Province, China
Supported by TCM Administration Bureau of Shannxi Province, China, No. 199704
Correspondence to: Dr. Yi Liu, Department of TCM, First Hospital of Xi'an Jiaotong University, Jiankang Road 1#, Xi'an 710061, Shaanxi Province, China. liuyi.jiaotong@163.com
Telephone: +86-29-81987592 Fax: +86-29-85252812
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Abstract

AIM: To study the inhibitory effects of Fuzheng Yiliuyin (Decoction for Suppressing Tumors by Strengthening the Body Resistance) in combination with chemotherapeutics on human gastric carcinoma cell strain.

METHODS: Fuzheng Yiliuyin (ZY) combined with various kinds of chemotherapeutics was put into two kinds of cultivated human gastric carcinoma cell strains, then its inhibitory effects on human gastric carcinoma cell strains were determind by the MTT method. Flow cytometer was used to assay the apoptosis rate, and the ultrastructure of gastric carcinoma cells was observed under transmission electron microscope.

RESULTS: Obvious apoptosis was seen in gastric carcinoma cells after treatment with ZY for 72 h. ZY and chemical drugs had synergistic inhibition effects on the cultivated gastric carcinoma cells, but the effects were different on various cell strains. The inhibitory effects of ZY could be strengthened by cytotoxic action and apoptosis. ZY combined with fluorouracil, etoposide and cisplatin (EFP) chemotherapeutics had better inhibitory effects on SGC-7901, while ZY combined with EFP or with DDP chemotherapeutics had better inhibitory effects than other drugs on MGC-803.

CONCLUSION: ZY induces apoptosis and inhibits the growth of gastric carcinoma cells. ZY has the synergistic function of chemotherapeutics.

Key words: Human gastric carcinoma cell strain; Traditional Chinese medicine; Chemotherapeutics

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INTRODUCTION

Fuzheng Yiliuyin (ZY) has been used for 30 years to treat gastric carcinoma in our clinic. ZY is prepared from eleven herbs: milkvetch root, bighead atractylodes rhizome, Chinese Thorowax root, common Burreed rhizome, Zedoary, Radix Notoginseng, Dandelion, spreading Hedyotis herb, Agrimony, Tamariskoid spikemoss herb and Glycyrrhizia. Most of these herbs can inhibit cell proliferation, induce apoptosis and have anti-cancer effects[1-4]. *In vitro* experiment has confirmed that Fuzheng Yiliuyin is effective in inhibiting proliferation of gastric cancer cells and induces apoptosis[5]. Further double-blind placebo-controlled trial of chemotherapy plus Fuzheng Yiliuyin/placebo in advanced stage gastric cancer showed that ZY has anti-gastric cancer effects with lower side effects[6]. Traditional Chinese medicines TCM could increase the effects and decrease the side effects of chemotherapeutics. However, whether TCM can increase all of the therapeutic effects of chemotherapeutics and whether the mechanism is the same remain unclear.

MATERIALS AND METHODS

Patients

The human gastric mucinous adenocarcinoma cell line (MGC-803) with lower differentiation was obtained from the Center of Molecular Biology of Xi'an Jioatong University, China. The human gastric adenocarcinoma cell line (SGC-7901) with lower metastasis was obtained from Xijing Hospital of the Fourth Military Medical University, China. Fuzheng Yiliuyin (ZY) extract contains 2 g/L crude herbs, which was produced by Xi'an Jioatong University. Fludrouracil (5-FU, Shanghai Pharmaceutical Factory), etoposide (Vp-16 Lianyungang Pharmaceutical Factory), cisplatin (DDP, Dezhou Pharmaceutical Factory), adriamycin (Pharmacia and Upjohn), annexin-V-FITC and PI (Roche), EDTA and MTT (Sigma) were used in the study.

Cell culture

Cells were cultured in RMPI1640 medium (pH 7.2-7.4)

Table 1 Effects of ZY in combination with chemotherapeutics on SGC-7901 MGC–803 cell proliferation (mean ± SD)

Group	SGC-7901			MGC–803		
	24 h	48 h	72 h	24 h	48 h	72 h
ZY	12.4 ± 7.9	17.4 ± 3.1	20.1 ± 5.5	8.9 ± 4.5	11.8 ± 5.4	14.8 ± 2.7
ZY + Vp-16	[a]51.2 ± 4.2	[c]56.9 ± 4.8	[c]62.3 ± 3.9	[g]36.9 ± 7.2	[i]48.9 ± 5.2	[k]58.8 ± 4.6
ZY + ADM	[a]46.5 ± 7.2	[c]50.1 ± 3.2	[c]55.4 ± 4.8	[g]34.5 ± 8.1	[i]43.6 ± 3.7	[k]50.7 ± 6.4
ZY + 5-Fu	[a]57.8 ± 2.6	[c]61.9 ± 6.3	[c]69.9 ± 8.4	[g]40.7 ± 6.6	[i]50.8 ± 4.7	[k]55.7 ± 3.2
ZY + DDP	[a]53.6 ± 2.4	[c]58.4 ± 1.7	[c]67.4 ± 5.7	[g]70.2 ± 3.5	[i]80.1 ± 3.6	[k]84.3 ± 7.5
ZY + EAP	[a]61.2 ± 3.3	[c]68.5 ± 3.9	[c]71.5 ± 3.4	[g]73.8 ± 4.6	[i]72.2 ± 4.1	[k]74.9 ± 3.4
ZY + EFP	[a]79.4 ± 2.8	[c]83.3 ± 4.8	[c]87.5 ± 4.3	[g]82.6 ± 7.8	[i]87.5 ± 3.1	[k]89.9 ± 4.8

[a]$P < 0.05$ *vs* 24 h, [c]$P < 0.05$ *vs* 48 h, [e]$P < 0.05$ *vs* 72 h, [g]$P < 0.05$ *vs* 24 h, [i]$P < 0.05$ *vs* 42 h, [k]$P < 0.05$ *vs* 72 h.

containing 100mL/L foetal calf serum (FCS), penicillin and streptomycin at 37℃ in a humidified (95%) incubator containing 95% air and (50mL/L) 5% CO_2. Cells were routinely sub-cultured using 2.5 g/L trypsin/ethylenedinitrile tetra-acetic acid (EDTA) solution.

MTT assay for determination of cell growth

Cell proliferation was determined by counting viable cells over time using trypan blue exclusion as the basis of viability. The logarithmically growing SGC-7901 and MGC-803 cells were plated at a density of 5×10^7 cells/L per well into a 96-well plate. After 24 h, the cells were treated with 1mg/L ZY in combination with 5-Fu, Vp-16, ADM, DDP, EFP and EAP, respectively for 24, 48, and 72 h. Control wells were treated with RMPI1640 medium alone. Then 20 μL MTT (5 g/L) was added to each well and incubated for an additional 4 h. After the culture media were discarded, 0.15 mL DMSO was added and vibrated for 10 min. The absorbance was measured at 570 nm using a Model 550-microplate reader. The inhibitory rates (IR) were calculated according to the following formula:

IR (%) = (1-absorbance of the treated wells)/(absorbance of the control wells) × 100%.

Flow cytometry of apoptosis by annexin-V and PI double-staining

At the end of treatment, the cells were harvested by trypsin-EDTA solution to produce single cell suspension. Annexin-V and PI double staining kit (Roche) were used to assess apoptosis. The cells were analyzed by flow cytometry. Early apoptotic cells were localized in the lower right quadrant of a dot-plot graph using annexin-V-fluorescein and PI.

Cell morphology observation

The SGC-7901 and MGC-803 cells were separately incubated with 1mg/L ZY for 72 h. The culture medium was discarded, the cells were collected by gently scraping and washed three times in ice-cold PBS, then fixed with a solution of 2% formaldehyde and 3% glutaraldehyde in 0.1 mol/L sodium cacodylate buffer (pH 7.4) for 1 h. The fixed cells were washed three times in cacodylate buffer (pH 7.2) containing 0.2 mol/L sucrose, fixed with 1% osmic acid in 0.3 mol/L cacodylate buffer, dehydrated in a graded series of acetone, and embedded in epoxy resin. Ultrathin sections were cut, stained with uranyl acetate and lead citrate, and assessed by electron microscopy

Table 2 Effects of ZY in combintion with chemotherapeutics on SGC-7901 MGC–803 cell apoptosis rates (mean ± SD)

	SGC-7901			MGC-803		
	alive	necrosis	apoptosis	alive	necrosis	apoptosis
ZY	78.7 ± 0.6	6.6 ± 4.3	14.5 ± 5.4	84.4 ± 3.1	4.5 ± 0.6	11.2 ± 1.8
ZY + Vp-16	38.5 ± 2.5	46.1 ± 6.2	15.3 ± 2.1	42.1 ± 1.5	32.4 ± 7.9	25.8 ± 5.2
ZY + ADM	44.6 ± 3.5	26.7 ± 2.5	19.9 ± 4.7	49.4 ± 5.2	29.8 ± 3.3	20.7 ± 5.1
ZY + 5-Fu	29.6 ± 4.1	44.7 ± 9.9	25.6 ± 1.2	44.3 ± 8.2	30.5 ± 7.4	25.3 ± 4.6
ZY + DDP	32.3 ± 11.7	63.6 ± 10.6	4.1 ± 3.0	16.1 ± 7.5	60.9 ± 8.1	23.0 ± 0.6
ZY + EAP	27.6 ± 5.8	55.5 ± 10.5	16.8 ± 7.4	25.0 ± 2.9	65.4 ± 5.8	9.6 ± 2.3
ZY + EFP	13.1 ± 2.7	66.3 ± 12.0	20.7 ± 6.6	11.1 ± 3.6	67.8 ± 4.1	20.9 ± 4.9
control	92.1 ± 6.3	6.6 ± 8.4	1.3 ± 0.2	94.3 ± 0.2	4.6 ± 0.8	1.2 ± 0.5

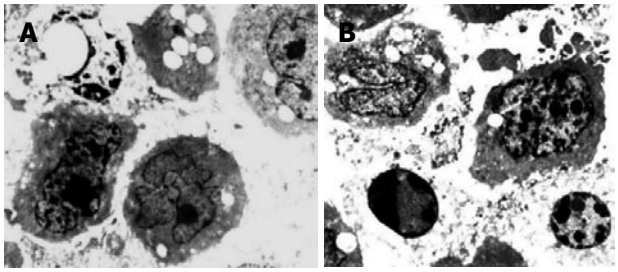

Figure 1 Morphological change of apoptosis in SGC-7901 (**A**) and MGC–803 (**B**) cells after treated with ZY.

Statistical analysis

Data were expressed as mean ± SD. Analysis of data was performed using one-way ANOVA. $P < 0.05$ was considered statistically significant.

RESULTS

SGC-7901 and MGC-803 cells were treated with various chemotherapeutics. Cell viability was determined by MTT assay. ZY increased the inhibitory effects of various chemotherapeutics on both kinds of cells in a time-dependent manner. ZY in combination with EFP had a better effect on SGC-7901 cells, while ZY in combination with DDP and EFP had a better effect on MGC-803 cells ($P < 0.05$).

After SGC-7901 and MGC-803 cells were exposed to ZY in combination with various chemotherapeutics for 72 h, annexin-V and PI double-staining FCM analysis showed that the apoptosis rates of SGC-7901 and MGC-803 cells were quite different after treated with different drugs. ZY increased anticancer effects of chemotherapeutics by directly enhancing the toxic effects or by inducing apoptosis (Table 2).

In order to confirm the cell apoptosis induced by treatment with ZY, the morphological changes of apoptosis were evaluated by transmission electron microscopy. Changes were observed in SGC-7901 cells (Figure 1A) and MGC-803 cells (Figure 1B) after treated with ZY, such as blebbing and loss of microvilli, vacuolation in cytoplasm, condensation of cytoplasm and nuclei, and fragmented

chromatin, which provided further evidence for the induction of apoptosis as a consequence of ZY treatment.

DISCUSSION

Gastric cancer is a common malignant tumor of the alimentary tract and its incidence is among the three leading kinds of neoplasm in different regions of China[7,8]. Conventional treatments for advanced gastric cancer including extended resection, radiotherapy and chemotherapy have little influence on the improvement of patients' survival. Combinations such as FAMTX, ELF, EAP and EFP, produce an overall response rate of about 20% to 50%[9-12]. Though combination chemotherapy for local advanced gastric cancer is effective, it is often associated with severe side effects, including fatal outcome.

Traditional Chinese herbal medicines can enhance the efficacy and reduce the toxicity of chemotherapy and radiotherapy by improving anticancer host defense mechanism and inducing apoptosis[13-19]. The use of Chinese herbal medicine in combination with radiotherapy or chemotherapy is a better procedure in the treatment of advanced cancer.

This study showed that Chinese herbal medicines could increase the effects of chemotherapy on gastric cancer, but the cytostatic effect of it is different between different differentiation cells. This is why the same Chinese herbal medicine in combination with the same chemotherapy is effective in some patients and less effective in other patients. Though combined chemotherapic agents may show a better sensitivity than a single agent, and various drugs show various effects on various alimentary tract tumors[20]. In this study, ZY in combination with a single chemolherapeutic agent or with several chemotherapies had the similar inhibitory effects on the same cells, suggesting that individual therapy should be performed based on the type and differentiation degree of tumors. Individual chemosensitivity is essential to chemotherapy for gastric cancer[21].

Apoptosis plays a critical role in tumor initiation, progression, as well as in cancer therapy[22]. In the present study, we investigated the inhibitory effects of ZY plus chemotherapy on gastric carcinoma SGC-7901 and MGC-803 cells. ZY caused not only cellular necrosis but also induced apoptosis and inhibited the proliferation of gastric cancer cells, indicating that TCM in combination with chemotherapy can induce apoptosis. In conclusion, ZY can be used in treatment of gastric cancer.

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