Effect of metformin combined with chemotherapeutic agents on gastric cancer cell line AGS

Xiaochang Wu
Department of General Surgery, Huzhou Central Hospital, Zhejiang University Huzhou Hospital, Zhejiang, China

Abstract: To explore the effect of metformin combined with chemotherapeutic agents on gastric cancer cell line AGS, 24 patients with gastric cancer were tracked for treatment. CCK-8, Transwell model and flow cytometry were used to detect the cell proliferation, migration ability and other indexes. The metformin inhibited the AGS cell proliferation in a dose- and time-dependent manner ($P<0.05$). The application of metformin, cisplatin, adriamycin or paclitaxel alone could effectively lower the migration and invasion ability of AGS cells. The metformin in combination with the three chemotherapeutic agents could effectively promote the apoptosis of AGS cells. The metformin in combination with chemotherapeutic agents can effectively and apparently treat the patients with gastric cancer, more significantly in clinic compared to the traditional administration mode. It can effectively promote the apoptosis of AGS cells, thus, it's worth adopting in clinic.

Keywords: Metformin, chemotherapeutic agents, human gastric cancer cell line AGS, clinical study.

INTRODUCTION

In China, gastric cancer is a kind of frequently-occurring malignant tumor in digestive tract, and its incidence is on rise every year and is ranked the top among tumor incidences (Uehara *et al.,* 2015). Especially, it is commonly-occurring in middle-aged and older people. At the early stage, its diagnosis rate is lower. Therefore, the gastric cancer, with higher death rate, can seriously endanger life (Xu *et al.,* 2010). Its occurrence is a complicated pathological process caused by multiple factors, such as environmental factors, intake of excess salt, smoking, drinking and imbalance of vitamins. Biotic factors such as long-term infection of *Helicobacter pylori*, genetic factors and mutations of cancer suppressor gene and carcinogenic gene (Nobes *et al.,* 2012; Shan *et al.,* 2015). As the cytokine was deeply studied, the correlation between occurrence and development of gastric cancer and body's AGS cells has been noted. We detected the human gastric adenocarcinoma cell (AGS) of gastric cancer patients and explored their role and significance in clinical treatment (Aljada *et al.,* 2012).

Relevant literature has reported that the endoscopy is more accurate and reliable for diagnosis of gastric cancer and its accuracy rate can be as high as 97.4% (Xu *et al.,* 2010). Radical operation should be adopted as much as possible, to prolong the patient's lifetime and guarantee life quality. If the radical operation is difficult, palliative gastrectomy can be performed. The palliative gastrectomy can effectively alleviate obstruction, bleeding, pain and other symptoms, and can lower cancer poisoning and immune loads, to prolong the lifetime, thus, advance prevention is essential (Nobes *et al.,* 2012). Many studies have shown that the metformin can reduce the incidence of cancer and effectively suppress the growth of tumor cells. The molecular mechanisms for several types of tumor cells are different. To obtain the more ideal curative effects in treating tumor in clinic, the dosage of chemotherapeutic agents must be increased, which will inevitably increase the toxic or side effects (Uehara *et al.,* 2015).

**Corresponding author:* e-mail: wuxiaochang16@163.com

MATERIALS AND METHODS

General data

24 gastric cancer patients that hospitalized in our hospital were verified to suffer from gastric cancer under gastro scope, biopsy, surgery and pathological section. All patients were free from any other systematic diseases, patients sample including 13 males and 11 females, with ages of 42-78 and averaging 53.6±7.1. All patients were approved by Ethics Committee of our hospital and signed on the informed consent.

Treatment methods

AGS cells were cultured in F-12K culture medium (100 U/mL of penicillin with volume fraction 10%FBS and 100mg/L of streptomycin) that was placed in an incubator with CO_2 of volume fraction 5% at 37^o. For the patients in the Metformin-alone group, different-concentration Metformin was used to treat the human's gastric cancer AGS cells, while for the patients in the chemotherapeutic agents group, 2mg of cisplatin, 0.02mg of adriamycin and 0.02mg of paclitaxel were used, respectively to treat the AGS cells. Then, Metformin in combination with cisplatin, adriamycin and paclitaxel was used to treat the AGS cells (Zhang *et al.,* 2013; Wu *et al.,* 2017). The expressions of cisplatin, adriamycin and paclitaxel on IC_{50} of AGS cell was 2, 0.02 and 0.02mg/L, respectively. All experiments were performed in triplicates.

Inhibition on cell proliferation

AGS cells at logarithmic growth phase were isolated and inoculated into a 96-hole plate after cell density was adjusted to 1×104 mL^{-1}.200μL/hole. After the cells adhered to the wall, the different-concentration Metformin (blank control group, 5.0, 10.0, 12.5, 17.5 and 20.0mmol/L) was used to treat the gastric cancer AGS cells, 6 duplicated holes for each concentration. After culturing for more 24h and 48h, 20μL of CCK-8 was added into each hole and incubated at 37^o for 2h. Afterwards, the enzyme-linked immune tester was used to measure the absorbance (A) at 450 nm. The proliferation inhibiting rate was (control A – experiment A)/control A ×100%.

Cell migration ability

After cultured for 24h, the AGS cells were resuspended with the culture medium (serum-free) containing 1g/L BSA for counting, 200ul of 1×105 mL^{-1} cell suspension was maintained in a 24-hole plate in a Transwell chamber. Afterwards, AGS cells were treated by groups, 500μL of 10% FBS culture solution was added into the lower chamber. After cells were migrated for 24 hours, the upper cells were wiped off, then fixed with formaldehyde (volume fraction 40%) for 20min and stained with 1g/L crystal violet for 15-20min, then inverted 200-fold microscope to count the number of cell-penetrating cells.

Invasion on cells

50 mg/L of Matrigel was diluted (1:5) and coated in the Transwell chamber. After culturing at 37^o for 3-4h, the residual liquid was absorbed after Matrigel turned into colloid.

Continuous apoptosis of cells

Cells were treated for 48h by groups, digested with pancreatin containing 20g/L BSA, centrifuged at 2 000 r/min for 5min. 1×106mL^{-1} suspension was prepared with Binding Buffer. Meanwhile, 500ul was extracted and added with 5μL of AnnexinV-FITC, incubated at room temperature (away from direct sunlight) for 15min, 5ul of PI was added, and incubated at room temperature (away from direct sunlight) for 15min. The flow cytometry was used for measurement and relevant software was used for comparison and analysis.

STATISTICAL ANALYSIS

SPSS 14.0 statistical software was used for statistical analysis. The experimental data were compared by factorial design, $P<0.05$ was considered a significant difference.

RESULTS

Suppression on cell proliferation

The results showed that metformin could inhibit AGS cell proliferation to a certain degree and was time-dependent and dosage-dependent (table 1), and the subsequent test in the experiments could be performed at this concentration, as shown in table 1.

Cell migration and invasion ability

As shown in the table 2, application of Metformin, cisplatin, adriamycin or paclitaxel alone had certain invasion on AGS cells in reduced trend, but the invasion of Metformin in combination with chemotherapeutic agents on AGS cells was not synergic. As shown in table 3, the application of Metformin, cisplatin, adriamycin or paclitaxel alone could increase the total percentage of apoptotic AGS cells, and Metformin in combination with cisplatin, adriamycin and paclitaxel had synergistic effects.

DISCUSSION

As the economical and technological progress, the prolonged average life expectancy of human continuously deepens the social aging degree and various geriatric diseases have been increasingly noted (Zhu *et al*., 2012; Tao *et al*., 2015). Gastric cancer is a kind of frequently-occurring malignant tumor and also a kind of common digestive system tumor in clinic. With higher death rate, it especially attacks the older people (Li *et al*., 2015). Currently, surgery is mainly performed for the aged gastric cancer patients. For most aged gastric cancer patients, their autoimmune function and metabolic function are lower, and physiological functions are reduced (Zheng *et al*., 2012). The aged gastric cancer patients suffer from various complications before operation and postoperative complications can easily occur, to bring great risks to operation and result in multiple problems for prognosis. With great changes of people's life style, the gastric cancer incidence is on increasing rise in recent years its incidence and death rate are higher in clinic (Xue *et al*., 2010). Relevant literatures have reported that the endoscopy is more accurate and reliable for diagnosis of gastric cancer its accuracy rate can be as high as 97.4% (Xu *et al*., 2010). Radical operation should be adopted as much as possible, to prolong the patient's lifetime and guarantee life quality. If the radical operation is difficult, palliative gastrectomy can be performed. The palliative gastrectomy can effectively alleviate obstruction, bleeding, pain and other symptoms, and can lower cancer poisoning and immune loads, to prolong the lifetime, so, in-advance prevention is essential.

Several studies have shown that the Metformin can reduce the incidence of cancer and effectively suppress the growth of tumor cells. The molecular mechanisms for different types of tumor cells are different. To obtain the more ideal curative effects in treating tumor in clinic, the dosage of chemotherapeutic agents must be increased, which will inevitably increase the toxic or side effects (Uehara *et al*., 2015). The Metformin as a kind of AMP-

Table 1: Metformin could inhibit AGS cell proliferation effect on cell proliferation

Concentration of Metformin (mmol·L^{-1})	24(h)	48(h)	72(h)
5	10.5±1.9	19.8±2.4	45.7±3.1
10	22.1±2.2	35.3±2.7	49.9±3.9
12.5	27.8±2.9	39.6±2.6	52.3±1.7
15	31.3±2.2	42.1±2.3	55.9±1.9

Table 2: Comparisons of Cell Migration with chemotherapeutic agents

Concentration of Metformin (mmol·L^{-1})	Count of cells if not combined with chemotherapeutic agents	Count of cells if combined with cisplatin	Count of cells if combined with adriamycin	Count of cells if combined with paclitaxel
0	170.5±34.9	119.8±19.4	93.5±14.3	83.2±11.7
10	101.3±18.2	72.4±15.3	60.9±11.9	52.9±10.0

Table 3: Comparison of Cell Invasion Ability with synergistic effects

Concentration of Metformin (mmol·L^{-1})	Count of cells if not combined with chemotherapeutic agents	Count of cells if combined with cisplatin	Count of cells if combined with adriamycin	Count of cells if combined with paclitaxel
0	110.4±10.9	81.7±8.4	75.5±7.3	69.2±7.7
10	68.3±9.2	50.3±8.3	46.9±6.9	39.1±5.8

Table 4: Comparison about Cell Apoptosis for apoptotic AGS cells

Concentration of Metformin (mmol·L－1)	Count of cells if not combined with chemotherapeutic agents	Count of cells if combined with cisplatin	Count of cells if combined with adriamycin	Count of cells if combined with paclitaxel
0	4.6±0.8	41.2±2.4	25.5±2.3	37.6.±2.7
10	38.3±1.5	50.1±8.3	46.9±6.9	48.1±2.1

activated protein kinase agonist, is a basic medicine in treating type 2 diabetes, with stable efficacy and safety. The Metformin can adjust AMPK pathway signal (Zhang *et al*., 2015). Clinically, to obtain an ideal efficacy in treating tumor, the dosage of chemotherapeutic agents must be increased, which will inevitably increase the adverse reactions.

CONCLUSION

The experimental results showed that the metformin inhibited the AGS cell proliferation in a dose dependent and time-dependent manner ($P<0.05$). The application of metformin, cisplatin, Adriamycin or paclitaxel alone could effectively lower the migration and invasion ability of AGS cells. The metformin in combination with the three chemotherapeutic agents could effectively promote the apoptosis of AGS cells.

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