

RESEARCH ARTICLE

Sensitivity of Gastric Cancer Cells to Chemotherapy Drugs in Elderly Patients and Its Correlation with Cyclooxygenase-2 Expression

Zhen-Qin Qiu¹, Zhen-Rong Qiu^{2*}

Abstract

Objective: To explore the sensitivity of gastric cancer cells to chemotherapy drugs in elderly patients and its correlation with cyclooxygenase-2 (COX-2) expression in cancer tissue. **Materials and Methods:** Forty-three elderly patients with gastric cancer (observation group) and 31 young patients with gastrointestinal tumors (control group) who were all diagnosed by pathology and underwent surgery in the 89th Hospital of Chinese People's Liberation Army were selected. Drug sensitivity testing of tumor cells in primary culture was carried out in both groups using a methyl thiazolyl tetrazolium (MTT) method, and the expression of COX-2 and the factors related to multi-drug resistance (MDR) in cancer tissue were assessed by immunohistochemistry. **Results:** The inhibition rates (IR) of vincristine (VCR), 5-fluorouracil (5-FU), oxaliplatin (L-OHP), mitomycin (MMC) and epirubicin (eADM) on tumor cells in the observation group were dramatically lower than in the control group, with statistical significance ($P < 0.05$ or $P < 0.01$). The positive rates of COX-2, glutathione s-transferase- π (GST- π) and P glycoprotein (P-gp) expression in cancer tissue in the observation group were all higher than in control group ($P < 0.05$), while that of DNA topoisomerase II α (TopoII α) expression lower than in the control group ($P < 0.01$). In the observation group, COX-2 expression in cancer tissue had a significantly-positive correlation with GST- π and P-gp ($r = 0.855, P = 0.000$; $r = 0.240, P = 0.026$), but a negative correlation with TopoII α ($r = -0.328, P = 0.002$). In the control group, COX-2 expression in cancer tissue was only correlated with P-gp positively ($r = 0.320, P = 0.011$). Bivariate correlation analysis displayed that COX-2 expression in cancer tissue in the observation group had a significantly-negative correlation with the IRs of 5-FU, L-OHP, paclitaxel (PTX) and eADM in tumor cells ($r = -0.723, P = 0.000$; $r = -0.570, P = 0.000$; $r = -0.919, P = 0.000$; $r = -0.781, P = 0.000$), but with hydroxycamptothecine (HCPT), VCR and 5-FU in the control group ($r = -0.915, P = 0.000$; $r = -0.890, P = 0.000$; $r = -0.949, P = 0.000$). **Conclusions:** Gastric cancer cells in elderly patients feature stronger MDR, which may be related to high COX-2 expression.

Keywords: Gastric cancer - chemotherapy drugs - cyclooxygenase-2 - glutathione s-transferase- π - P glycoprotein -

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Introduction

Gastric cancer, one of the most common malignant tumors, is the leading cause for cancer-related deaths around the world (Fagoonee et al., 2014). The patients with gastric cancer have few or mild symptoms at an early stage and the lesions have been at the advanced stage when typical symptoms appear (Lu et al., 2013). The occurrence and progression of gastric cancer is a multi-step and multi-stage process involved in various oncogenes, such as proto-oncogenes, tumor suppressor genes, pro-apoptotic genes and apoptosis suppressor genes (He et al., 2013). Chemotherapy plays a pivotal role in the comprehensive treatment of gastric cancer. Nevertheless, multi-drug resistance (MDR) is one of the major causes

that lead to chemotherapy failure. The deaths of over 90% cancer patients treated with chemotherapy are caused by MDR (Tsuruo et al., 2003). Therefore, to seek MDR formation mechanism and effective reversal agents has been a challenge in the field of cancer research.

Drug resistance produced by tumor cells results from interaction of various drug-resistant mechanisms, in which glycoprotein-mediated MDR is one of the dominant action mechanisms. As an inducible enzyme, cyclooxygenase-2 (COX-2) participates in tumorigenesis and progression. Its expression is up-regulated under the stimulation of cytokines, growth factors and oncogenes (Hua et al., 2015; Misron et al., 2015), and its high expression can promote tumor angiogenesis via up-regulation of angiogenesis factors in tumor cells. Besides, it also takes part in tumor

¹Unified Supply Center, ²Pharmacy Department, The 89th Hospital of Chinese People's Liberation Army, Weifang, China *For correspondence: qzqsxg@163.com

formation in gastrointestinal system through a lot of pathways (Shao et al., 2014; Sierra et al., 2013; Wang et al., 2014). In recent years, the studies have confirmed that COX-2 is closely related to MDR, and its reversal agents can effectively reverse MDR phenotypes (Liu et al., 2009). The elderly patients have their own characteristics in terms of some tumor cell MDR, and hence, proper chemotherapy regimens should be selected according to their characteristics. Nowadays, there are no reports about the sensitivity of gastric cancer cells to chemotherapy drugs in elderly patients and their correlation with COX-2 expression in cancer tissue. In this study, the sensitivity of gastric cancer cells to chemotherapy drugs in vitro was analyzed, and the expression of COX-2 and MDR-related factors was detected so as to provide more clinical evidences for reversing MDR of gastric cancer cells in elderly patients.

Materials and Methods

General data

Research objects: Forty-three elderly patients with gastric cancer diagnosed by pathology who underwent surgery in The 89th Hospital of Chinese People's Liberation Army were selected as observation group, in which the males and females were 29 and 14 cases, respectively. They were 62~78 years old, averagely (67.5±6.6) years old. Meanwhile, 31 young patients with gastrointestinal tumors diagnosed by pathology who underwent surgery were selected as control group at the same term, in which there were 21 males and 10 females. They were at the age of 42~58, with the mean age of (49.4±6.2). All patients were not treated with chemotherapy before surgery. Both primary lesions and corresponding lesions with lymph node metastasis of each sample were fixed using 10% neutral formalin first, and then embedded with paraffin and cut into slices. Finally, pathological diagnosis and classification were conducted through hematoxylin-eosin (HE) staining.

Drugs and relevant reagents: Ten commonly-encountered chemotherapy drugs were selected, including hydroxycamptothecine (HCPT), vincristine (VCR), 5-fluorouracil (5-FU), cisplatin (DDP), oxaliplatin (L-OHP), paclitaxel (PTX), etoposide (VP-16), mitomycin (MMC), epirubicin (eADM) and pirarubicin (THP). Culture solution (DMEM medium) and cell culture plate were provided by Shanghai Hengyuan Biological Technology Co., Ltd. Mouse anti-human COX-2, glutathione s-transferase- π (GST- π), P glycoprotein (P-gp) and DNA topoisomerase II α (TopoII α) monoclonal antibodies as well as relevant immunohistochemical kits were all purchased from Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd.

Methods

Drug sensitivity test of tumor cell primary culture: The drug sensitivity test was carried out in 2 h after fresh removed cancer tissues (0.5cm × 0.5cm × 1.0cm) were placed in double-antibody culture solution. The concentration of cancer tissue was regulated into 5×10^5 /mL with a cell separator, and then 10 sorts of

chemotherapy drugs were respectively set 2 parallel holes to make their final concentrations reach the peak in plasma (Tan et al., 2010). Cell suspension was inoculated using a drug sensitivity detection plate first (200 μ L per hole), and then vibrated and mixed up by a micro-oscillator, finally placed in a 37°C, 5% CO₂ incubator for incubation 48 h. After that, 5 mg/mL of methyl thiazolyl tetrazolium (MTT) was added, 20 μ L per hole, for continuous incubation 3~24 h. The culture was terminated if MTT formed into blue acicular crystals under the microscope. The supernate was discarded first after a cell culture plate was used to centrifuge 10 min in 1 000 r/min; Next, dimethyl sulfoxide (DMSO) was added, 100 μ L per hole, then vibrated slightly and mixed up; At last, the optical density (OD) value was measured using 570 nm microplate reader. Average inhibition rate (IR) of tumor cells = (1-average OD value of medication hole/average OD value of control hole) × 100%.

Immunohistochemical staining: COX-2 and MDR-related factors in cancer tissue (GST- π , P-gp and TopoII α proteins) were stained using immunohistochemistry. The staining was operated strictly according to kit instructions.

Judging criteria

Two clinical pathologists observed the sections in a double-blind method, randomly selected 5 high-power visual fields (×400) in each section and calculated 100 cells in every visual field. The positive expression of COX-2, P-gp and GST- π as well as TopoII α proteins was respectively defined as presence of claybank granules in tumor cytoplasm, cytoplasm or cytomembrane and cell nucleuses.

Staining gradings were separated according to staining degrees of tumor cells and percentage of positive cells. The scoring criteria for staining degrees: 0 point (colourless), 1 point (light yellow), 2 points (yellow) and 3 points (claybank). The scoring criteria for percentage of positive cells: 0 point (the percentage of positive cells ≤5%), 1 point (the percentage being 6%~25%), 2 points (the percentage being 26%~50%), 3 points (the percentage being 51%~75%) and 4 points (the percentage ≥76%). The product of two sorts of scores above was regarded as the final score, namely 0~1 point being negative (-), 2~3 points being weakly positive (+), 4~7 points being positive (++) and ≥8 points being strongly positive (+++).

Table 1. Comparison on the IR of 10 Chemotherapy Drugs on Tumor Cells in Two Groups ($\bar{x} \pm s$, %)

Drugs	Observation group (n=43)	Control group (n=31)	t	P
HCPT	29.79±10.10	30.72±9.59	0.3991	>0.05
VCR	26.78±8.71	31.51±9.92	2.1742	<0.05
5-FU	27.19±6.06	32.31±7.98	3.1380	<0.01
DDP	27.72±9.30	26.90±7.92	0.3977	>0.05
L-OHP	27.69±10.72	33.62±12.93	2.1527	<0.05
PTX	45.64±12.52	44.56±11.80	0.3749	>0.05
VP-16	25.59±7.18	26.03±9.81	0.2229	>0.05
MMC	17.60±7.37	23.47±8.60	3.1513	<0.01
eADM	24.51±11.67	31.58±10.63	2.6677	<0.01
THP	23.59±9.32	26.69±12.90	1.2010	>0.05

Comparison on the expression of COX-2 and MDR-related factors in cancer tissue

Table 2. Comparison on the Expression of COX-2 and MDR-Related Factors in Cancer Tissue[n (%)]

Indexes		Observation group (n=43)	Control group (n=31)
COX-2	-	8 (18.6)	13 (41.9)
	+	13 (30.2)	7 (22.6)
	++	2 (4.7)	5 (16.1)
	+++	20 (46.5)*	6 (19.4)
GST- π	-	2 (4.7)	3 (9.7)
	+	4 (9.3)	5 (16.1)
	++	17 (39.5)	16 (51.6)
	+++	20 (46.5)*	7 (22.6)
P-gp	-	2 (4.7)	4 (12.9)
	+	5 (11.6)	6 (19.4)
	++	12 (27.9)	11 (35.5)
	+++	24 (55.8)*	10 (32.2)
TopoII α	-	3 (7.0)	1 (3.2)
	+	15 (34.9)	5 (16.1)
	++	18 (41.9)	10 (32.2)
	+++	7 (16.3)**	15 (48.4)

Compared with control group, * $P < 0.05$, ** $P < 0.01$.

Statistical data analysis

SPSS 15.0 statistical software was used to analyze the data. Measurement data were compared with t test and expressed by mean \pm standard deviation ($\bar{x} \pm s$); Ranked data were compared with Wilcoxon paired rank-sum test and bivariate correlation analysis. $P < 0.05$ was considered to be statistically significant.

Results

Comparison on the IR of 10 chemotherapy drugs on tumor cells in two groups

Among 10 commonly-encountered chemotherapy drugs, the IR of VCR, 5-FU, L-OHP, MMC and eADM on tumor cells in observation group were dramatically lower than in control group, with statistical significance ($P < 0.05$ or $P < 0.01$) (Table 1).

The positive rates of COX-2, GST- π and P-gp expression in cancer tissue in observation group were all higher than in control group dramatically ($P < 0.05$), while that of TopoII α expression lower than in control group ($P < 0.01$) (Table 2).

Relationship between the expression of COX-2 and MDR-related factors in cancer tissue

Bivariate correlation analysis revealed that in observation group, COX-2 expression in cancer tissue had a significantly-positive correlation with GST- π and P-gp ($r = 0.855$, $P = 0.000$; $r = 0.240$, $P = 0.026$), but a negative correlation with TopoII α ($r = -0.328$, $P = 0.002$). In control group, COX-2 expression in cancer tissue was only correlated with P-gp positively ($r = 0.320$, $P = 0.011$), not correlated with GST- π and TopoII α significantly ($r = 0.133$, $P = 0.302$; $r = 0.093$, $P = 0.471$).

Correlation of COX-2 expression in cancer tissue with IR of chemotherapy drugs on tumor cells

Bivariate correlation analysis displayed that COX-2 expression in cancer tissue in observation group had a

significantly-negative correlation with the IR of 5-FU, L-OHP, PTX and eADM on tumor cells ($r = -0.723$, $P = 0.000$; $r = -0.570$, $P = 0.000$; $r = -0.919$, $P = 0.000$; $r = -0.781$, $P = 0.000$), but only with HCPT, VCR and 5-FU in control group ($r = -0.915$, $P = 0.000$; $r = -0.890$, $P = 0.000$; $r = -0.949$, $P = 0.000$).

Discussion

Gastric cancer is one of the malignant tumors with the highest incidence and mortality in China (Liu et al., 2013; Wei et al., 2013; Xu et al., 2013). Its prognosis is poorer and 5-year survival rate is only 15%~20% (Li et al., 2015). The study demonstrated that adjuvant chemotherapy for gastric cancer after surgery, especially for advanced gastric cancer, could effectively remove micrometastases and ameliorate the patients' prognosis (Basaran et al., 2015). Nevertheless, MDR produced by tumor cells can decrease the chemotherapeutic effect conspicuously. About 30~80% tumor cells are drug-resistant in the process of chemotherapy, consequently leading to chemotherapy failure.

Klepin et al. found that the elderly patients with acute myeloid leukemia had stronger drug-resistance to chemotherapy drugs and had their own characteristics for MDR in some tumors (Klepin et al., 2009). The results in this study revealed that the IR of VCR, 5-FU, L-OHP, MMC and eADM on tumor cells in observation group were dramatically lower than in control group, indicating that the patients' age is related to the drug-resistance of tumor cells to chemotherapy drugs, and the drug-resistance of elderly patients is stronger. Additionally, the expression of GST- π , P-gp and TopoII α was also detected to further investigate the drug-resistant mechanism. All of GST- π , P-gp and TopoII α belong to typical MDR-related factors, and their-induced MDR phenotypes are the markers of acquired or intrinsic drug-resistance. By different pathways including binding to enzymes and drug-pump effect, both GST- π and P-gp can make a variety of chemotherapy drugs discharge from the body to induce MDR (Zhang et al., 2009). As a crucial target in the chemotherapy, up-regulation of TopoII α expression can reinforce the sensitivity of tumor cells to chemotherapy drugs (Shi et al., 2008). The results in this study displayed that the positive rates of GST- π and P-gp expression in cancer tissue in observation group were all higher than in control group dramatically, while that of TopoII α expression lower than in control group, suggesting that the tumor cells in elderly patients can act on the chemotherapy drugs via a lot of pathways and make them lose efficacy, thereby affecting the chemotherapeutic effect severely.

In recent years, nonsteroidal anti-inflammatory drugs dominated by aspirin play important roles in the prevention and treatment of tumors, especially in gastrointestinal tumors. One of the possible mechanisms for preventing and treating tumors is to reduce the generation of prostaglandins by inhibiting the key enzymes that synthesize COX. As a key rate-limiting enzyme in the biological synthesis of prostaglandins in vivo, COX falls into two types, namely COX-1 and COX-2. COX-2 expression goes up abnormally in most colon

cancer and gastric cancer tissues as well as cell lines, but application of COX-2 inhibitors can effectively inhibit the proliferation of tumor cells (Xu et al., 2014). Some studies showed that COX-2 inhibitors in combination with the chemotherapy drugs, such as adriamycin and 5-FU, could dramatically reinforce the sensitivity of gastric cancer cells to chemotherapy drugs, suggesting that COX-2 inhibitors probably have sensibilization to chemotherapy drugs (Zhu et al., 2007). Chemotherapy drugs can decrease their own induction effect on gastric cancer cells by increase of COX-2 expression, consequently resulting in MDR of tumor cells to chemotherapy. The results in this study revealed that the positive rate of COX-2 expression in cancer tissue in observation group was evidently higher than in control group. Besides, COX-2 expression in cancer tissue in observation group had a significantly-negative correlation with the IR of 5-FU, L-OHP, PTX and eADM on tumor cells, and there were no drugs whose IR on tumor cells was positively correlated with COX-2 expression. All these results indicated that COX-2 could induce the MDR of tumor cells by modulating the expression of some MDR-related factors, suggesting that in elderly patients, MDR-related factors modulated by COX-2 in cancer tissue and their involved drug-resistant pathway may be different from young patients, so the drug-resistance in cancer tissue is stronger.

The studies have also confirmed that COX-2 can modulate the expression of GST- π , P-gp and TopoII α (Segawa et al., 2008; Sui et al., 2011). The results in this study displayed that in observation group, COX-2 expression in cancer tissue had a significantly-positive correlation with GST- π and P-gp, but a negative correlation with TopoII α . However, in control group, COX-2 expression in cancer tissue was only correlated with P-gp positively, displaying that COX-2 expression is associated with the MDR of tumor cells, and its high expression may be one of the enhanced drug-resistant reasons in elderly patients.

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