



# Effect of preoperative oral S-1 combined with regional intra-arterial chemotherapy on malignant molecule expression in locally advanced unresectable gastric cancer tissue

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## ABSTRACT

**Objective:** To study the effect of preoperative oral S-1 combined with regional intra-arterial chemotherapy on malignant molecule expression in locally advanced unresectable gastric cancer tissue. **Methods:** A total of 144 patients with locally advanced gastric cancer receiving surgical resection after neoadjuvant chemotherapy in our hospital between May 2012 and August 2015 were selected and randomly divided into experimental group who received preoperative oral S-1 combined with regional intra-arterial chemotherapy and control group who received preoperative intravenous systemic chemotherapy. The levels of serum tumor markers were determined after chemotherapy, and the expression levels of tumor suppressor genes and cell cycle-related molecules in tumor tissue were determined after surgical resection. **Results:** After neoadjuvant chemotherapy, the serum G-17, TK-1, CEA, CA19-9, CA12-5, CA72-4 and CK, CK-MB, ALT, AST levels of experimental group were significantly lower than those of control group; after surgical resection, the *p16*, *p27*, *PTEN* and *TXNIP* mRNA levels in tumor tissue of experimental group were significantly higher than those of control group while *CyclinB2*, *CyclinD1*, *CyclinE*, *CDK1* and *CDK2* mRNA levels were significantly lower than those of control group. **Conclusions:** Preoperative oral S-1 combined with regional intra-arterial chemotherapy can more effectively kill gastric cancer cells, reduce tumor load, inhibit cell cycle and promote cell apoptosis.

## 1. Introduction

Gastric cancer is one of the malignant tumors of digestive system with the highest incidence, and the new cases of gastric cancer each year in China account for more than 50% of the new cases of gastric cancer in the world. At present, the early diagnostic rate of gastric cancer is not high, most patients with gastric cancer are in advanced stage at the time of diagnosis, and some patients are with locally advanced unresectable gastric cancer. In clinical practice, killing cancer cells, reducing tumor volume and reducing tumor grading by neoadjuvant chemotherapy can seek the opportunity of surgical

resection for patients with locally advanced gastric cancer[1,2]. Intravenous systemic chemotherapy solution mFOLFOX6 is the most common neoadjuvant chemotherapy method for advanced gastric carcinoma, and although it can effectively kill gastric cancer cells, it will lead to different degree of adverse reactions and affect the patients' tolerance to surgical resection, and is greatly limited in clinical application[3,4]. S-1 is a newly developed anticancer drug, it can metabolize in the body and produce 5-fluorouracil and delay its metabolism, and its chemotherapy effect is exact; regional intra-arterial chemotherapy can improve the concentration of chemotherapeutics within the lesion and enhance the killing effect on cancer cells[5]. In the following study, the effect of preoperative oral S-1 combined with regional intra-arterial chemotherapy on malignant molecule expression in locally advanced unresectable gastric cancer tissue was analyzed.

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## 2. Materials and methods

### 2.1. Research subjects

A total of 144 patients with locally advanced gastric cancer receiving neoadjuvant chemotherapy and surgical resection after chemotherapy in our hospital between May 2012 and August 2015 were selected, were all diagnosed with gastric cancer by pathological examination, were with infiltration depth of T3-T4 and without distant metastases confirmed by the imaging examination, and did not receive radiotherapy and chemotherapy. Rule out exist distant organ metastasis and patients with peritoneal spread planting transfer. After signing informed consent, they were randomly divided into experimental group ( $n=72$ ) who received preoperative oral S-1 combined with regional intra-arterial chemotherapy and control group ( $n=72$ ) who received preoperative intravenous systemic chemotherapy. Experimental group included 48 male cases and 24 female cases, they were ( $53.7\pm 7.4$ ) years old, 39 cases were with infiltration depth of T3 and 33 cases were with T4; control group included 46 male cases and 26 female cases, they were ( $55.1\pm 7.8$ ) years old, 41 cases were with infiltration depth of T3 and 31 cases were with T4. The two groups of patients showed no significant difference in general information.

### 2.2. Chemotherapy

Neoadjuvant chemotherapy of experimental group was as follows: they received oral administration of S-1 capsules 40 mg, 2 times/day, on the 1-14 day, 21 days as a cycle; they received regional intra-arterial chemotherapy on each of the 14th day of the treatment cycle, Seldinger method was used for super-selective femoral artery intubation to the supply artery of tumor, then oxaliplatin  $100\text{ mg/m}^2$  and epirubicin  $30\text{ mg/m}^2$  were injected, 1/2 of the dose was injected at first, and the other 1/2 of the dose was mixed with 40% iodized oil emulsion and then injected to embolize the artery. Control group received chemotherapy as per mFOLFOX6

scheme, which was as follows: oxaliplatin  $100\text{ mg/m}^2$ , intravenous drip, on the 1st day, leucovorin  $400\text{ mg/m}^2$ , intravenous drip, on the 1st day, 5-fluorouracil  $2400\text{ mg/m}^2$ , intravenous drip for 46 hours. They received surgical resection 3-4 weeks after neoadjuvant chemotherapy.

### 2.3. Detection methods of indexes in serum

After neoadjuvant chemotherapy, peripheral blood specimens were collected from the two groups and centrifuged to get serum, enzyme-linked immunosorbent assay kits were used to determine gastrin-17 (G-17), thymidine kinase-1 (TK-1), carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9), CA12-5 and CA72-4 levels, and full-automatic biochemical analyzer was used to determine CK, CK - MB, ALT and AST levels.

### 2.4. Detection methods of gene expression in tumor tissue

After surgical resection of gastric cancer, tumor tissue was collected, washed with saline for 2-3 times and then added in Trizol lysis buffer to extract RNA in the tissue, reverse transcription kits were used to synthesize the first strand of cDNA, then the fluorescence quantitative PCR amplification was conducted, amplified target genes included *p16*, *p27*, *PTEN*, *TXNIP*, *CyclinB2*, *CyclinD1*, *CyclinE*, *CDK1* and *CDK2*, and internal reference gene was  $\beta$ -actin. The mRNA levels of target genes were calculated according to the amplification curve.

### 2.5. Statistical methods

SPSS20.0 software was used to input and analyze data, measurement data analysis was performed by *t* test and  $P<0.05$  indicated statistical significant differences.

## 3. Results

**Table 1**

Comparison of serum tumor marker levels between two groups.

Groups	Case No.	G-17(pmole/L)	TK-1(pmole/L)	CEA( $\mu\text{g/L}$ )	CA19-9(U/mL)	CA12-5(U/mL)	CA72-4(U/mL)
Experimental group	72	12.5 $\pm$ 2.1	1.42 $\pm$ 0.20	9.5 $\pm$ 1.0	20.3 $\pm$ 3.2	28.6 $\pm$ 4.1	7.61 $\pm$ 0.13
Control group	72	19.4 $\pm$ 2.9	2.15 $\pm$ 0.31	17.6 $\pm$ 2.4	36.6 $\pm$ 4.9	55.2 $\pm$ 7.8	15.42 $\pm$ 1.96
<i>t</i>		7.387	7.039	8.685	8.118	9.582	11.472
<i>P</i>		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

**Table 2**

Comparison of serum myocardial injury and liver damage indexes between two groups (U/L).

Groups	Case No.	CK	CK-MB	ALT	AST
Experimental group	72	64.59 $\pm$ 9.35	3.37 $\pm$ 0.58	42.58 $\pm$ 6.84	39.34 $\pm$ 5.39
Control group	72	96.57 $\pm$ 11.37	7.37 $\pm$ 0.84	72.35 $\pm$ 8.94	63.58 $\pm$ 8.24
<i>t</i>		7.182	11.382	8.958	9.332
<i>P</i>		<0.05	<0.05	<0.05	<0.05

### 3.1. Serum tumor marker levels

Serum G-17, TK-1, CEA, CA19-9, CA12-5 and CA72-4 levels of experimental group were significantly lower than those of control group ( $P < 0.05$ ) (Table 1).

### 3.2. Serum myocardial injury and liver damage indexes

Serum CK, CK-MB, ALT and AST levels of experimental group were significantly lower than those of control group ( $P < 0.05$ ) (Table 2).

### 3.3. Tumor suppressor gene expression in tumor tissue

*p16*, *p27*, *PTEN* and *TXNIP* mRNA levels in tumor tissue of experimental group were significantly higher than those of control group ( $P < 0.05$ ) (Table 3).

**Table 3**

Comparison of tumor suppressor gene expression levels in tumor tissue between two groups ( $\beta$ -actin).

Groups	Case No.	<i>p16</i>	<i>p27</i>	<i>PTEN</i>	<i>TXNIP</i>
Experimental group	72	5.83±0.85	7.14±0.89	2.57±0.33	3.89±0.52
Control group	72	3.25±0.47	2.89±0.35	1.04±0.15	2.14±0.29
<i>t</i>		7.699	15.498	13.328	8.627
<i>P</i>		<0.05	<0.05	<0.05	<0.05

### 3.4. Cell cycle-related molecule expression in tumor tissue

*CyclinB2*, *CyclinD1*, *CyclinE*, *CDK1* and *CDK2* mRNA levels in tumor tissue of experimental group were significantly lower than those of control group ( $P < 0.05$ ) (Table 4).

## 4. Discussion

Locally advanced unresectable gastric cancer is the clinical common type of gastric cancer, a significant proportion of patients with gastric cancer have already progressed to advanced stage at the time of diagnosis and missed the opportunity of surgical resection, and neoadjuvant chemotherapy is needed at this time to strive for the opportunity of surgical resection for patients[6]. mFOLFOX6 is the common neoadjuvant chemotherapy for advanced gastric cancer, but intravenous systemic chemotherapy can cause strong adverse reaction, some patients can't tolerate the surgical treatment because of the strong adverse reaction, and some patients are unable to

complete chemotherapy because of the strong adverse reaction[7,8]. As a result, intravenous mFOLFOX6 chemotherapy has limited clinical application value. Oral S-1 combined with regional intra-arterial chemotherapy is a new neoadjuvant chemotherapy regimen developed in recent years. Study has shown the regimen is more safe and effective, the adverse reaction in patients with advanced gastric cancer is lighter after oral S-1 combined with regional intra-arterial chemotherapy, and the histopathologic curative effect is more satisfactory after surgical resection[9]. In the study, myocardial injury indexes and liver damage indexes were used to reflect the safety of the chemotherapy regimen, and the result showed that serum CK, CK-MB, ALT and AST levels of experimental group after chemotherapy were significantly lower than the control group. This means that oral S-1 combined with regional intra-arterial chemotherapy is with weaker cardiotoxicity and hepatotoxicity, and its security is superior to that of traditional intravenous systemic chemotherapy.

The ingredients of S-1 include tegafur, gimeracil and potassium oxonate, tegafur can enter the body and then be metabolized into 5-fluorouracil (5-Fu) and exert killing effect on cancer cells, gimeracil can inhibit the activity of 5-Fu metabolism enzyme, hinder 5-Fu metabolism and increase the concentration of 5-Fu in the body, and potassium oxonate can act on the orotate transphosphoribosylase in intestinal tract, block the phosphorylation of 5-Fu, reduce its absorption within the gastrointestinal tract and reduce gastrointestinal adverse reactions[10–12]. Regional intra-arterial chemotherapy can make chemotherapeutics directly enter into the supply artery of tumor lesions, significantly increase the concentration of chemotherapeutics within the lesions and enhance the killing effect of chemotherapeutics on cancer cells; what's more, the application of embolism agent can further cause of interruption blood supply within the lesions and cause anoxic injury to cancer cells, and can also prolong the continuous duration of chemotherapeutics within the lesions[13,14]. In order to define the effect of oral S-1 combined with regional intra-arterial chemotherapy, the levels of serum tumor markers were detected at first in the study to evaluate the tumor load after chemotherapy, thus reflecting the killing effect of two chemotherapy regimens on gastric cancer cells. The analysis of the tumor marker content showed that serum G-17, TK-1, CEA, CA19-9, CA12-5 and CA72-4 levels of experimental group were significantly lower than those of control group. This means that oral S-1 combined with regional intra-arterial chemotherapy has better killing effect on gastric cancer cells than traditional intravenous systemic chemotherapy, and the tumor load after chemotherapy is

**Table 4**

Comparison of cell cycle-related molecule expression levels in tumor tissue between two groups ( $\beta$ -actin).

Groups	Case No.	<i>CyclinB2</i>	<i>CyclinD1</i>	<i>CyclinE</i>	<i>CDK1</i>	<i>CDK2</i>
Experimental group	72	5.69±0.84	3.59±0.52	7.14±0.95	2.42±0.38	1.89±0.24
Control group	72	9.14±1.17	8.26±0.94	13.54±1.88	5.79±0.85	3.24±0.46
<i>t</i>		8.389	13.582	9.581	12.578	8.185
<i>P</i>		<0.05	<0.05	<0.05	<0.05	<0.05

lower.

In the growth process of advanced gastric cancer lesions, infinite proliferation of cancer cells is the most important biological basis, and the deletion or decrease of the expression of a variety of tumor suppressor genes is associated with the enhancement of the cancer cell proliferation ability. *p16*, *p27*, *PTEN* and *TXNIP* are the several tumor suppressor genes known to be with inhibitory effect on cell proliferation. The products encoded by *p27* gene and *p16* gene have inhibitory effect on many kinds of cell cycle-dependent kinases, and can block the cell cycle progression from G<sub>1</sub> phase to S phase, thereby inhibiting the cell cycle development and cell proliferation[15,16]; the protein encoded by *PTEN* gene can inhibit PI3K/AKT signal pathway through dephosphorylation effect, thereby inhibiting the cell proliferation mediated by signaling pathway[17]; *TXNIP* is a member of the  $\alpha$ -arrestin protein family, and can activate apoptosis signal-regulating kinase 1, promote cell apoptosis and inhibit cell proliferation. In the study, analysis of the expression levels of above tumor suppressor genes in tumor tissue proved that *p16*, *p27*, *PTEN* and *TXNIP* levels in tumor tissue of experimental group were significantly higher than those of control group. This means that oral S-1 combined with regional intra-arterial chemotherapy can increase the expression of tumor suppressor genes in lesion tissue, thus promoting the apoptosis of cancer cells. The development of cell cycle is the biological basis of cell proliferation, and the interactions between Cyclin and Cyclin-dependent kinase (CDK) can shorten the cell cycle duration and accelerate cell cycle transition, thus promoting cell proliferation. In the study, analysis of Cyclin and CDK expression levels in tumor tissue showed that *CyclinB2*, *CyclinD1*, *CyclinE*, *CDK1* and *CDK2* levels in tumor tissue of experimental group were significantly lower than those of control group. This means that oral S-1 combined with regional intra-arterial chemotherapy can inhibit the expression of Cyclin and CDK in lesion tissue, thus inhibiting the proliferation of cancer cells. To sum up, preoperative oral S-1 combined with regional intra-arterial chemotherapy can more effectively kill gastric cancer cells, reduce tumor load, inhibit cell cycle and promote cell apoptosis.

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