

# The effect and clinical efficacy of lienal polypeptide injection combined with FOLFOX chemotherapy regimen in colon cancer patients

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**Abstract.** The aim was to investigate the clinical efficacy and immunomodulatory effect of lienal polypeptide injection combined with postoperative FOLFOX chemotherapy regimen in colon cancer patients. A total of 84 colon cancer patients were selected between January, 2014 and December, 2015. The selected patients were randomly divided into the observation (42 patients) and control (42 patients) groups. The observation group patients were treated with FOLFOX chemotherapy regimen combined with lienal polypeptide, whereas, the control group patients were treated with FOLFOX chemotherapeutic regimen only. After two cycles of treatment, Karnofsky performance scale (KPS) index, side effects or toxicity and the peripheral blood T-cell subset analyses in the two groups of patients were evaluated. The patients who received FOLFOX chemotherapeutic regimen combined with lienal polypeptide in the observation group showed significantly higher score of KPS than patients of the control group ( $P<0.05$ ). No significant difference in the spectrum of side effects such as nausea, vomiting, neurotoxicity, liver and kidney dysfunction, oral mucosal inflammation were observed in the treatment groups. However, the incidence of bone marrow suppression was significantly lower in the observation group in comparison with the control group ( $P<0.05$ ). Additionally, the CD8<sup>+</sup> T cells were decreased in the observation group patients compared to the control group, while the ratio of CD4<sup>+</sup> T/CD8<sup>+</sup> T ( $T_H/T_C$ ) cells and the number of natural killer cells were higher in the observation group patients than the control group ( $P<0.05$ ). In conclusion, the results suggest that, when the standard FOLFOX chemotherapeutic regimen is combined with lienal polypeptide to treat

colon cancer, it enhances the general well being of patients and strengthens the immune system in the combat against cancer.

## Introduction

Non-communicable diseases contribute to 83% of deaths and of these 21% are due to cancer in China (1). Colon cancer is one of the most common types of cancer in many countries with an incidence of 1,360,000 cases and mortality of 694,000 individuals, but 3,544,000 cancer survivors worldwide (2). A recent estimate suggested that, China has an incidence of 253,000 cases and mortality rate of 139,000 cases of colon cancer and these rates show a rising trend (2). Currently, the comprehensive treatment is dominated by surgery followed by radiotherapy, chemotherapy and other adjuvant therapy. Surgical resection is the preferred method of treatment for colon cancer because of its effectiveness (3). However, in the majority of patients, surgery does not arrest the risk of relapse. Consequently, adjuvant chemotherapy to patients that have undergone surgery is an efficacious method for preventing relapse and metastasis and an improved prognosis in clinical settings (4).

A majority of patients were in the mid-late stage when initially diagnosed, while approximately 60-70% of patients require adjuvant chemotherapy after surgery. The 'FOLFOX4' program is one of the main chemotherapeutic regimens for patients having undergone surgery for colon cancer (5). FOLFOX4 contains the drugs oxaliplatin, leucovorin (LV), and 400 and 600 mg<sup>2</sup> fluorouracil. Oxaliplatin is a third generation platinum anticancer drug, which regards DNA as the target site where the platinum atoms and DNA strand forms interstrand and intrastrand crosslink, thereby blocking DNA replication and transcription. Oxaliplatin is also non-cross-resistant with cisplatin, thus producing a synergistic effect in combination with fluorouracil (6).

The National Comprehensive Cancer Network and other guidelines clearly suggest that, for stage III colon cancer patients after surgery, any of the following adjuvant chemotherapeutic regimens including the FOLFOX program, Xelox program, or 5-Fu/CF program should be used (7). However, for stage II colon cancer patients after surgery, no recommendation is available regarding the use of adjuvant therapy, whereas

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for high-risk stage II colon cancer patients after surgery, adjuvant chemotherapy is recommended only during conditions including T4 (stage IIB), histological grading (grade III or IV), vascular tumor thrombus, preoperative intestinal obstruction or perforation, lymph node detection number <12 or no clear resection margin (8,9). The recommended duration of post-operative adjuvant chemotherapy was 6 months.

Lienal polypeptide injection is manufactured from healthy calf spleen extract and 1 ml contains 4 mg of polypeptide, 5 mg of free amino acid, 1 mg of nucleic acid, and not <100  $\mu$ g of total sugar. Previously it was shown that, the administration of calf spleen extract significantly improved viscera index, phagocytosis index, NK cell killing activity, IgG level in serum and elevated peripheral blood leukocyte and lymphocyte counts in immunocompromised mice (10). Previous findings showed that, lienal polypeptide can function as an immune modulator and effectively enhanced the immunity of patients (11,12).

The aim of the present study was to investigate the immunomodulatory effect and clinical efficacy of FOLFOX4 chemotherapeutic regimen combined with lienal polypeptide in colon cancer patients.

### Patients and methods

**Patients.** In total, 84 pathologically confirmed patients who underwent surgery for colon cancer in the Department of Medical Oncology of Xuzhou Central Hospital (Jiangsu, China) from January, 2014 to December, 2015 were included for the study. Of the 84 patients, 56 were men and 28 women, with a median age of  $52.8 \pm 6.8$  years. The patients of the present study were required to be pathologically diagnosed to have colon cancer, to be in stage II or III with high risk, to have routine blood, liver and kidney function tests and ECG performed, to have no contraindications for chemotherapy, expected to have survival for more >3 months and to be ready to receive chemotherapy four weeks after surgery. The patients used mandarin to communicate and had no severe cognitive impairment, and no history of psychiatric, alcohol and drug dependence. The clinicopathological characteristics of the two groups are shown in Table I.

The patients were randomly divided into the observation and control groups using the random number method. After four weeks of surgery, patients in the observation group (42 patients) received adjuvant FOLFOX4 chemotherapy combined with lienal polypeptide. The remaining 42 patients in the control group were administered only with FOLFOX4 adjuvant chemotherapeutic regimen.

The present study complied with the Declaration of Helsinki and was approved by the Ethics Committee of Central Hospital of Xuzhou. Written informed consent was obtained from all the patients.

**Treatment of patients.** The observation group patients received FOLFOX4 chemotherapeutic regimen, which consisted of L-OHP 85 mg/m<sup>2</sup> intravenous infusion 2 h on day 1, CF 200 mg/m<sup>2</sup> intravenous infusion 2 h on days 1-2, 5-Fu 400 mg/m<sup>2</sup> intravenous infusion on days 1-2, and 5-Fu 600 mg/m<sup>2</sup> continuous trace chemotherapy pump infusion 22 h on days 1-2. A portable continuous intravenous infusion pump was used (2C1009K, 5 ml/h velocity; Baxter, Deerfield,

Table I. Clinicopathological characteristics of the patients.

Parameters	Observation group	Control group	P-value
Gender			0.81
Male	29	27	
Female	13	15	
Age (years)			0.59
$\leq 40$	2	1	
41-50	17	18	
51-60	14	16	
$\geq 60$	9	7	
Median age	$52.3 \pm 7.1$	$53.1 \pm 6.6$	
Pathological type			0.78
Adenocarcinoma	38	37	
Non-adenocarcinoma	4	5	
TNM staging			0.87
II	12	14	
III	30	28	
Pre-chemotherapy KPS score			0.49
70	8	7	
80	21	24	
90	13	11	
Median KPS score	$86.3 \pm 7.1$	$85.6 \pm 6.9$	

KPS, Karnofsky performance scale.

IL, USA). Concomitantly, the observation group patients were administered with 0.9% sodium chloride injection 500 ml plus 10 ml of lienal polypeptide (2/100 ml; Jilin Fengsheng Pharmaceutical Co. Ltd., Jilin, China) intravenous infusion once a day on days 1-10. The observation and control group patients were treated with FOLFOX4 regimen and FOLFOX4 combined with lienal polypeptide, respectively, for two cycles and each consisted of 14 days. Blood routine, liver and kidney functions were evaluated before and after chemotherapy of each cycle. Direct immunofluorescence labeling, whole blood hemolysis method and flow cytometry were used to detect lymphocytes and its subsets and these analyses were carried out 1-2 days before the first chemotherapy and 7-8 days after completion of the two cycles of chemotherapy.

**Karnofsky performance scale (KPS) index.** The KPS was used to determine the score (10-100) that was optimal to the patient's activity at a particular time-point to evaluate functional status of the patient after completion of two cycles of chemotherapeutic regimen.

**Toxicity and side effects.** Side effects such as nausea, vomiting, bone marrow suppression, neurotoxicity, the impairment of liver and renal function and oral mucosal inflammation after chemotherapy were recorded. The data were graded according to the grading standards for the toxicity of chemotherapeutic drugs (WHO).

Table II. Incidence of side effects during chemotherapy.

Groups	No. of patients	Nausea, vomiting, n (%)	Bone marrow suppression, n (%)	Neurotoxicity, n (%)	Liver function impairment, n (%)	Kidney function impairment, n (%)	Oral mucosal inflammation, n (%)
Observation	42	23 (55)	17 (40) <sup>a</sup>	18 (43)	2 (5)	0 (0)	5 (12)
Control	42	28 (67)	29 (69)	20 (48)	3 (7)	1 (2)	6 (14)
Total	84	51 (61)	46 (55)	38 (45)	5 (6)	1 (1)	11 (13)

<sup>a</sup>P<0.05 compared with the control group.

Table III. T-cell subsets between the observation and control groups before and after chemotherapy.

T-cell subsets	Observation group		Control group	
	Before	After	Before	After
CD4 <sup>+</sup> T (%)	30.52±4.77	31.24±4.51	30.15±3.64	30.52±4.26
CD8 <sup>+</sup> T (%)	28.26±4.10	20.38±3.55 <sup>a,b</sup>	28.48±3.98	24.97±3.48
CD4 <sup>+</sup> T/CD8 <sup>+</sup> T	1.21±0.41	1.73±2.99 <sup>a,b</sup>	1.19±0.36	1.41±3.08 <sup>a</sup>
NK cells (%)	9.16±3.72	13.27±4.22 <sup>a,b</sup>	9.27±4.19	9.36±4.56

<sup>a</sup>P<0.05 compared with before chemotherapy. <sup>b</sup>P<0.05 compared with the control group.

**Peripheral blood T-cell subset analysis.** Prior to and after chemotherapy, peripheral blood T-cell subset analyses were carried out in the two groups of patients. The analyses included CD4<sup>+</sup> lymphocytes (T<sub>H</sub> lymphocytes), CD8<sup>+</sup> lymphocyte (T<sub>S</sub> lymphocytes), NK cells and the ratio of CD4<sup>+</sup>/CD8<sup>+</sup>.

**Statistical analysis.** SPSS 17.0 statistical software (Chicago, IL, USA) was used for the statistical analysis. The  $\chi^2$  test was used to compare the KPS score and toxicity between two groups before and after chemotherapy. The independent samples t-test was used to compare the changes of CD4<sup>+</sup> cells, CD8<sup>+</sup> cells, NK cell activity and CD4<sup>+</sup>/CD8<sup>+</sup> of peripheral blood between two groups of patients before and after chemotherapy. P<0.05 was considered statistically significant.

## Results

**KPS and side effects.** The average KPS score of the observation group patients was significantly higher (85.9±6.5) in comparison with the control group patients (74.3±5.9) and this difference was statistically significant (P<0.05). The observed side effects in the two groups of patients were nausea, vomiting, bone marrow suppression, peripheral neurotoxicity (manifested as limb numbness, cold stimulation aggravation, and relief after withdrawal) and oral mucosal inflammation (grade I). These side effects were similarly distributed between the two groups, with the exception of bone marrow suppression. Bone marrow suppression was observed in only 40% of patients of the observation group, whereas, 69% of the control group patients showed bone marrow suppression (P<0.05; Table II).

**Effect of treatment on immune cells.** Before chemotherapy, the number of CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells, NK cells and the ratio of CD4<sup>+</sup>/CD8<sup>+</sup> showed no significant difference between the two groups of patients (P>0.05).

Within each group, the level of peripheral blood CD8<sup>+</sup> T cells prior to chemotherapy was decreased in comparison with levels after chemotherapy, while the ratio of CD4<sup>+</sup>/CD8<sup>+</sup> after chemotherapy was significantly increased (P<0.05) in comparison with the levels prior to chemotherapy. However, the observation group (FOLFOX + lienal polypeptide) showed a significantly higher ratio of CD4<sup>+</sup>/CD8<sup>+</sup> cells and NK cells compared to the control group (P<0.05; Table III).

## Discussion

In China, the incidence and mortality of colon cancer have increased significantly in the last 30 years, and the age of onset has also significantly advanced. The 5-year survival rate after colon cancer surgery in limitation period was 70%, for stage III patients while it was 30-60 and 7% for patients in advanced stage (13). Therefore, the timely detection of colon cancer, immediate surgical intervention, followed by postoperative adjuvant chemotherapy according to the post-operative pathology and staging, can effectively increase the survival rate and quality of life of patients (14).

Postoperative adjuvant chemotherapy affects biosynthesis and interferes with the normal metabolism of tumor cells, thereby inhibiting tumor cell proliferation. Subsequently, tumor cell killing is achieved. Thus, tumor relapse can be prevented and patient prognosis improved. The mechanism of adjuvant chemotherapy is dependent on chemotherapy and

is able to regulate residual lesions after radical surgery (15). After surgical resection, the tumor cell load is small and the sensitivity of distantly metastasized tumor cells or local residual tumor cells to chemotherapy is high. Additionally, adjuvant chemotherapy is highly advantageous in the reduction of the formation of drug-resistant cancer cell clones (16). Thus, the combination of 5-Fu with LV chemotherapeutic regimen is considered as the classical treatment for colorectal cancer (17).

As an immune modulator, the lienal polypeptide has a dual regulatory effect on the immune system of the body. It corrects the disorder of immune function, thereby activating and enhancing non-specific immune function, promotes the maturation of T lymphocytes and sensitizes lymphocytes to improve the immune function of lymphocytes. The lienal polypeptide also induces the production of interferons that directly block viral protein synthesis and replication, and enhances the antigen presentation on the cell surface, promotes the cytotoxic activity of NK cells, and regulates lymphocyte and macrophage function, thus significantly improving the cell-mediated immune function. It also stimulates proliferation of bone marrow cells to produce a large number of white blood cells and enhances hematopoietic function (18). In addition, lienal polypeptide inhibits cell glycolysis without non-target cytotoxicity, in order that the high degree of glycolysis, an important feature of tumor cells, is reduced. This reduction results in the arrest of tumor cell metabolism and the prevention of tumor cells from entering into the G<sub>1</sub> phase, thus producing an anticancer effect (19).

The present findings indicate that, in the observation group, the post-treatment KPS score was significantly higher than that of the control group, and myelosuppression was obviously reduced suggesting that, lienal polypeptide stimulated the proliferation of bone marrow cells and promoted the recovery of hematopoietic function of bone marrow. Additionally, the ratio of CD4<sup>+</sup>/CD8<sup>+</sup> and the number of NK cells were increased in the observation group patients compared to the control group and this revealed that lienal polypeptide significantly improved the immune function, and reduced the undesired effect of chemotherapy on the immune function along with improvement in the quality of life of the patients.

From the present study, it may be concluded that, for post-operative colon cancer patients, the combination of standard FOLFOX chemotherapy with lienal polypeptide can significantly alleviate bone marrow inhibition, enhances the immune function of patients and improves the quality of life and is worthy of further research and application.

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