THERAPEUTIC EFFECTS OF COMBINATION OF CHEMOTHERAPY AND BIOTHERAPY ON COLORECTAL CANCER AND ITS EFFECTS ON IMMUNE CELLS, NK, IFN-γ AND IL-2

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ABSTRACT

Purpose: *To investigate the therapeutic effects of chemotherapy combined with biotherapy on colorectal cancer (CRC) and its effects on immune cells, NK, IFN-γ and IL-2.*

Methods: *Seventy patients with CRC treated in our hospital from March 2015 to July 2017 were selected and randomly divided into observation group and control group. The control group was treated with XELOX chemotherapy regimen, while the observation group received XELOX chemotherapy combined with dendritic cell (DC)-cytokine-induced killer cell (CIK) immunotherapy. Immune cells, peripheral blood cytokines, clinical efficacy, quality of life and the incidence of adverse reactions were compared between the two groups before and after treatment.*

Results: *After treatment, the levels of $CD3^+$, $CD3^+/CD4^+$, $CD3^+/CD8^+$, NK and CIK cells in observation group were significantly increased and higher than those in the control group, while their corresponding levels in the control group were significantly decreased ($p < 0.05$). The levels of IFN-γ and IL-2 in the observation group were significantly increased after treatment and significantly higher than those in the control group after treatment ($p < 0.05$). There was no significant difference in IL-6 in the observation group before and after treatment, but it was significantly lower than that of the control group after treatment ($p < 0.05$). The levels of IFN-γ and IL-2 in the observation group decreased significantly after treatment, while IL-6 in the observation group was significantly increased ($p < 0.05$). Effectiveness in the observation group was 60.00 %, and the rate of control was 85.71 %. These were significantly higher than the corresponding values in control group (31.43 % and 51.43 %, respectively) ($p < 0.05$). The quality of life in the observation group was 85.71 %, which was significantly higher than that in the control group (54.29 %) ($p < 0.05$). Adverse reactions such as peripheral neurotoxicity, bone marrow suppression, nausea and diarrhea in the observation group were significantly lower than those in the control group ($p < 0.05$).*

Conclusion: *Chemotherapy combined with biotherapy improves immune function in patients with colorectal cancer, increases their serum NK, IFN-γ and IL-2 levels, improves clinical effectiveness and quality of life, and reduces the incidence of adverse reactions.*

Keywords: *Colorectal cancer, Biotherapy, Immunotherapy, DC-CIK cell.*

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Introduction

Colorectal cancer (CRC) is one of the most common malignant tumors of the digestive tract[1]. In the clinical treatment of CRC, surgical resection is the main method used, while radiotherapy and chemotherapy are used as adjuvant therapy to improve the survival rate of the patients[2]. However, about 25 % of the patients have obvious metastasis at the time of diagnosis, and so cannot benefit from surgery. Moreover, the toxic and side effects of radiotherapy and chemotherapy can damage the immune function of the patients and affect their quality of life[3]. At present, biotherapy has become one of the most important methods for treating malignant tumors. The DC-CIK cell immunotherapy is an important part of biotherapy. The DC cells are the main antigen-presenting cells in humans which can effectively resist tumor cell resistance and initiate antigen T cell immune response. The CIK cells are cytotoxic T cells induced by human peripheral blood mononuclear

cells (PBMC) in vitro, and they have both non-MHC restriction and T lymphocyte tumor-killing activity[4]. Therefore, DC-CIK cells have strong tumor antigen-presenting ability and highly effective killing activity which can stimulate autoimmune system activity and enhance the anti-tumor ability of the patients. They are widely used in the treatment of malignant tumors because of their high anti-tumor activity, wide range of tumor-killing effects, low toxic and side effects, and safety in clinical use[5]. In the present study, one group of patients with CRC admitted and treated in our hospital from March 2015 to July 2017 were treated with XELOX chemotherapy combined with DC-CIK cell immunotherapy, while another group received XELOX chemotherapy. This study was aimed at investigating the therapeutic effects of chemotherapy combined with biotherapy on colorectal cancer as well as its effects on immune cells, NK, IFN-γ and IL-2.

Clinical Data and Methods

General informations

A total of 70 patients with CRC who were admitted and treated in our hospital from March 2015 to July 2017 were selected and examined by colonoscopy and pathology, and they met the diagnostic criteria of CRC[6]. Inclusion criteria: Patients who satisfied the following criteria were included: Clinical stage II, III or IV; Karnofsky score (KPS) equal to or greater than 70; expected survival time greater than 6 months; absence of serious diseases of heart, lung, liver and kidney; and absence of serious virus and bacterial infections. Exclusion criteria: patients in the following category were excluded: patients who did not tolerate the chemotherapy regimen; subjects allergic to biological products; patients with autoimmune diseases; patients with severe coagulation dysfunction, and those with mental illnesses. The patients were divided into observation group and control group using the random number table method. The general clinical data of the two groups were basically comparable, as shown in Table 1.

The study was approved by the local ethics committee and informed consent was signed by all patients. The control group was treated with XELOX regimen and the observation group was treated with XELOX chemotherapy combined with DC-CIK cell immunotherapy.

Clinical data		Observation group (n=35)	Control group (n=35)	χ^2	*p*
Sex	Male	23	25	0.265	0.607
	Female	12	10		
Pathological type	Adenocarcinoma	33	32	0.215	0.643
	Other diseases	2	3		
Clinical stages	Stage II	2	5	1.429	0.232
	Stage III	29	27	0.357	0.550
	Stage IV	4	3	0.159	0.690
Degree of differentiation	High	7	6	0.95	0.759
	Moderate	18	17	0.057	0.811
	Low	10	12	0.265	0.607
Occurrence site	Rectum	22	20	0.238	0.626
	Colon	13	15		

Table. 1: General clinical data of patients.

Main reagents and instruments

Flow cytometer was purchased from BD Company, USA, while CO_2 incubator was obtained from Shanghai Qiao Yue Electronic Technology Co., Ltd. Other instruments and their sources were: Spectra blood component separator (Gambro BCT company, USA); enzyme linked immune-surgery instrument (Jinan Baihe Medical device Co., Ltd); lymphocyte separation medium (Beijing Solarbio Technology Co., Ltd.); T-lymphocyte subgroup detection kit (Shanghai Caiyou Industry Co., Ltd), and ELISA kits for IFN-γ, IL-2 and IL-6 (Shanghai Qi Ming Biotechnology Co., Ltd.).

DC-CIK cell culture

Peripheral blood mononuclear cells (PBMC) were obtained using blood component separator. The cell density was adjusted to 2×10^6 cells/mL and the cells were incubated at 37 °C in a 5 % CO_2 incubator for 1 h.

DC cell culture: The cells were removed, and GM-CSF and IL-4 were added on day 1. The culture medium was changed once every 3 days; tu-

mor antigen-loaded DC cells were added on day 5; TNF-α was added on the 6th day to induce the DC cells to mature; and mature DC cells were harvested on the 7th day.

CIK cell culture: The cell concentration was adjusted to 2×10^6 cells/mL with serum-free medium, and IFN-γ was added on the first day, while CD3-activated monoclonal antibody and IL-2 were added on the second day. Culture medium was changed once every 3 days, and CIK cells were obtained on the 7th day.

DC and CIK cells co-culture: The mature DC and CIK cells were mixed and co-cultured at 1:10 ratio. The culture medium was changed once every 3 days, and IL-2 was added and co-cultured for 6 days.

Cell infusion

Bacteria, fungi, endotoxin and mycoplasma were detected 2 days before cell infusion, and cells were collected after the test results were negative. After washing with sterile normal saline for 3 times, the cells were suspended in 200 mL of normal saline containing 2% albumin. The number of cells was more than 5.0×109. The cells were infused intravenously once a day and the infusion was completed in 3 days.

Therapeutic regimen

The control group received oxaliplatin (130 mg/m^2) intravenous drip 2 h on the first day. Then, 1000 mg/m^2 of capecitabine was taken orally twice a day from day 1 to day 14, with 21 days as 1 cycle of chemotherapy. The chemotherapy ranged from 4 cycles to 6 cycles according to the patient's condition. In the observation group, peripheral blood were collected 2 days before chemotherapy for CIK-DC cell culture, and were infused for 3 days from day 14 to day 16. The patients received a total of four courses of CIK-DC cell therapy.

Observation and detection indices

The observation indexes included clinical effectiveness, quality of life, adverse reactions and immune function. The clinical effectiveness was with reference to the response evaluation criteria in solid tumors (RECIST)[7]. Computed tomography (CT) and B ultrasound were used for comparison one month before and after treatment. The curative effect was evaluated in terms of complete remission (CR), partial remission (PR), stable (SD) and progression (PD). Percentage effectiveness was calculated this:

$$\textit{Effectiveness}\ (\%) = \frac{(CR+PR)}{Total} \times 100$$

$$\textit{Effectiveness}\ (\%)\ \text{in Control} = \frac{(CR+PR+SD)}{Total} \times 100$$

Life quality evaluation:

According to KPS score[8], improvement in life quality $= \frac{(\text{improvement + stability})}{Total} \times 100$

Incidence of adverse reactions: According to the acute and subacute toxic reactions and grading standard set by National Cancer Institute (NCI) of the United States[9], adverse reactions are categorized into 0 - IV degrees. Changes in peripheral neurotoxicity, bone marrow suppression, gastrointestinal reaction, blood routine and liver function were observed before and after treatment.

Immune function test: Venous blood (5mL) was taken from patients with CRC 1 week before and after treatment. The positive expressions of total T cells (CD3+), Th cells (CD3+/CD4+), Tc cells (CD3+/CD8+), NK cells (CD3+/CD56+), CIK cells (CD3+/CD56+), Treg cells (CD4+/CD25+) in T cell subsets were determined with flow cytometry. The levels of IFN-γ, IL-2 and IL-6 in serum were assayed using ELISA method according to the kit instructions.

Statistical Analysis

The data obtained in this study were subjected to statistical analyses using SPSS21.0 software. The counting data are expressed as percentage, and χ^2 test was used for comparison. Measurement data are presented as mean ± standard deviation (SD) ($\bar{x}\pm s$), and were compared using t-test. Differences were considered statistically significant at the test level of $p < 0.05$.

Results

Changes of T cell subsets

The results showed that there was no significant difference in T cell subsets between the two groups before treatment ($p>0.05$). However, after treatment, CD3+, CD3+/CD4+, CD3+/CD8+, NK and CIK cells in the observation group were increased, and were significantly higher than those in the control group ($p<0.05$). The number of Treg cells changed in the observation group, but there

was no significant difference (p > 0.05). The levels of CD3+, CD3+/CD4+, CD3+/CD8+ and NK cells in the control group were decreased significantly (p<0.05). There were slight but non-significant decreases in CIK and Treg cells (p>0.05). These results are shown in Table 2.

Group	Time	CD3+	CD3+ /CD4+	CD3+ /CD8+	NK	CIK	Treg
Observation (*n*=35)	Before treatment	66.78± 17.51	37.95± 11.43	25.93± 12.02	11.37± 3.34	7.68± 2.45	6.48± 2.01
	After treatment	77.62± 13.13*	43.41± 10.29*	33.22± 11.17*	14.63± 4.07*	12.51± 4.72*	6.03± 1.57
Control (*n*=35)	Before treatment	65.42± 17.27	36.68± 10.92	27.64± 10.37	12.45± 4.66	8.13± 1.64	5.91± 1.84
	After treatment	51.36± 11.47*	22.34± 9.03*	20.19± 7.58*	9.28± 3.74*	7.39± 1.73	6.32± 2.25
T (observation group after treatment VS control group after treatment)		8.911	9.105	5.711	5.726	6.026	0.625
P (observation group after treatment VS control group after treatment)		0.001	0.001	0.001	0.001	0.001	0.534

Table. 2: T-cell subsets in the two groups (%, $\bar{x}\pm s$).
**p <0.05, compared with the same group before treatment.*

Changes in cytokines in peripheral blood

The results showed that there was no significant difference in cytokines between the two groups before treatment (p>0.05). After treatment, the levels of IFN-γ and IL-2 in the observation group were significantly higher than those before treatment, and were significantly higher than those in the control group (p<0.05). There was no significant difference in IL-6 in the observation group before and after treatment (p>0.05), but it was significantly lower than that in the control group after treatment (p<0.05). The levels of IFN-γ and IL-2 in the observation group after treatment were significantly lower than those before treatment (p<0.05). In addition, IL-6 was significantly increased (p<0.05). These results are displayed in Table 3.

Group	Time	IFN-γ	IL-2	IL-6
Observation (*n*=35)	Before treatment	15.96±2.51	13.12±2.56	6.87±1.92
	After treatment	23.07±3.28*	18.73±3.49*	7.86±2.95
Control (*n*=35)	Before treatment	15.74±2.12	12.36±2.83	6.59±1.47
	After treatment	11.53±1.89*	7.94±1.38*	9.65±1.34*
T (observation group after treatment VS control group after treatment)		18.035	17.001	3.268
P (observation group after treatment VS control group after treatment)		0.001	0.001	0.002

Table. 3: Changes in cytokines in peripheral blood of patients in the two groups before and after treatment (ng/L, $\bar{x}\pm s$).
**p <0.05, compared with the same group before treatment.*

Clinical effectiveness in the two groups

As shown in Table 4, after treatment, the effectiveness in the observation group was 60.00% and the degree of control was 85.71 %. These were significantly higher than the corresponding values in control group (31.43% and 51.43%, respectively) (p<0.05).

Group	Cases	CR	PR	SD	PD	Effectiveness	Degree of control
Observation	35	2 (5.71%)	19 (54.29%)	9 (25.71%)	5 (14.29%)	21 (60.00%)	30 (85.71%)
Control	35	1 (2.86%)	10 (28.57%)	7 (20.00%)	17 (48.57%)	11 (31.43%)	18 (51.43%)
χ^2	-	0.348	4.769	0.324	9.546	5.757	9.546
P	-	0.555	0.029	0.569	0.002	0.016	0.002

Table. 4: Comparison of clinical efficacy evaluation (%).

Quality of life evaluation

After treatment, the increase in quality of life in the observation group was 85.71 %, which was significantly higher than that in the control group (54.29 %) (p < 0.05), as shown in Table 5.

Group	Cases	Improvement	Stabilization	Worsening	Degree of increase
Observation	35	16 (45.71%)	14 (40%)	5 (14.29%)	30 (85.71%)
Control	35	12 (34.29%)	7 (20 %)	16 (45.71%)	19 (54.29%)
χ^2	-	0.952	3.333	8.231	8.231
P	-	0.329	0.068	0.004	0.004

Table. 5: Quality of life evaluation (%).

Adverse reactions

The incidents of adverse reactions in the two groups were compared and the results showed that peripheral neurotoxicity, bone marrow depression, nausea and diarrhea in the observation group were significantly lower in the observation group than in the control group ($p<0.05$). There were no significant differences in the incidents of liver dysfunction and fever between the two groups ($p>0.05$). These results are shown in Table 6.

Group	Cases	Peripheral neurotoxicity	Bone marrow depression	Nausea	Diarrhea	Liver dysfunction	Fever
Observation	35	15 (42.86%)	28 (80%)	27 (77.14%)	18 (51.43%)	9 (25.71%)	4 (11.43%)
Control	35	6 (17.14%)	16 (45.71%)	11 (31.43%)	7 (20%)	7 (20%)	3 (8.57%)
χ^2	-	5.51	8.811	14.737	7.529	0.324	0.159
P	-	0.019	0.003	0.001	0.006	0.569	0.690

Table. 6: Comparison of adverse reactions between the two groups.

Discussion

Colorectal cancer (CRC) is one of the most common malignant tumors of digestive tract in China. Surgical resection combined with postoperative adjuvant chemotherapy is commonly used in the treatment of CRC. However, the toxicity and side effects of chemotherapy are high. Thus, the patient's immune system is damaged after long-term chemotherapy, which makes it necessary to combine immunotherapy with chemotherapy so as to improve the curative effect. The occurrence, development and metastasis of tumor are closely related to the immune status of patients. The CD8+ cells inhibit T cells, and Treg cells participate in immune escape, and also inhibit the proliferation and activation of CD4+ CD8+ cells [10]. Therefore, the determination of T cell subsets is an important index for evaluating the immune function of patients. The results of this study indicate that CD3+, CD3+/CD4+, CD3+/CD8+, NK and CIK cells in the observation group were increased and significantly higher than those in the control group. The CD3+, CD3+/CD4+, CD3+/CD8+ and NK cells were decreased significantly in control group. These results suggest that chemotherapy reduces the immunity of patients, but combination of chemotherapy and immunotherapy reduces the immunosuppression caused by chemotherapy and improves the immune function of patients.

Studies have shown that there is a decrease in DC cells and dysfunction in most tumor patients, suggesting that it is possible to induce a sufficient number of viable DC cells to correct the immune deficiency in patients in vitro {3, 11]. The CIK cells secret a large number of cytokines (IFN-γ and IL-2); enhance cellular immunity, and secrete a mass of IL-6 cytokines to enhance cytotoxicity[12-14]. After treatment, the levels of IFN-γ and IL-2 in the observation group were significantly higher than those before treatment and significantly higher than those of the control group, and IL-6 was significantly lower than its level in the control group after treatment. In the observation group, IFN-γ and IL-2 decreased significantly, while IL-6 increased significantly, when compared with values before treatment. These findings indicate that DC-CIK immunotherapy can stimulate T cells to secrete cytokines, improve the cellular immune function of patients, and improve the anti-tumor effect.

The effectiveness in the observation group was 60.00 % and the degree of control was 85.71 %, which was significantly higher than that of the control group (31.43 and 51.43%, respectively). The increase in quality of life in the observation group was 85.71 %, which was significantly higher than that in the control group (54.29 %). The incidents of adverse reactions such as peripheral neurotoxicity, bone marrow depression, nausea and diarrhea in the observation group were significantly lower than those in the control group. These results indicate that combined therapy can significantly improve clinical effectiveness, reduce adverse reactions and improve the quality of life of patients.

Conclusion

These results strongly suggest that XELOX combined with DC-CIK regimen is more effective than XELOX regimen alone in the treatment of CRC. It can significantly improve the cellular immunity of patients, increase serum NK, IFN-γ and IL-2 levels, enhance the clinical effectiveness and improve the life quality of patients by reduce the incidents of adverse reactions. The regimen has good application value and is worthy of clinical application.

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