Journal of Hainan Medical University

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Influence of DC−CIK in advanced colorectal cancer patients on T lymphocyte subsets and cytokines

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ARTICLE INFO

Article history:
Received 5 May 2016
Received in revised form 16 May 2016
Accepted 15 May 2016
Available online 28 May 2016

Keywords:
Dendritic cells
Cytokine induced killer cells
Colorectal cancer
T lymphocyte subsets
Cytokines

ABSTRACT

Objective: To explore the effects of dendritic cells (DC)-cytokine induced killer cells (CIK) treatment on T lymphocyte subsets and cytokines in patients with advanced colorectal cancer. **Methods:** A total of 84 cases patients with advanced colorectal cancer were divided into two groups according to random number table method, each 42 cases, both two groups were given FOLFOX scheme chemotherapy, on the basis, the observation group were given supplementary DC-CIK treatment, compared the T lymphocy te subgroup: $CD3^+$, $CD4^+$, $CD8^+$, $CD4^+/CD8^+$, Th1 cytokines, interleukin-2 (IL-2) and interferon gamma-γ (FN-γ), Th2 cytokines: interleukin 6 (IL-6), interleukin 4 (IL-4) of the two groups before and after treatment. **Results:** Compared with before treatment, the $CD3^+$, $CD4^+$, $CD8^+$, $CD4^+/CD8^+$, Th1 cytokines IL-2 and IFN-γ in observation group were significantly higher than after treatment , the $CD3^+$, $CD4^+$, $CD8^+$, $CD4^+/CD8^+$, Th1 cytokines IL-2 and IFN-γ in control group were significantly lower than after treatment, and the differences were all statistically significant; The $CD3^+$, $CD4^+$, $CD8^+$, $CD4^+/CD8^+$, Th1 cytokines IL-2 and IFN-γ in observation group after treatment were significantly higher than those in control group after treatment with statistical difference; $CD8^+$, Th2 cytokines IL-4, IL-6 in two groups had no statistical significance before and after treatment. **Conclusion:** Chemotherapy can cause the immune function restrained in patients with advanced colorectal cancer, and DC-CIK supplementary therapy can significantly improve the immune function, enhance the anti tumor immune responses.

1. Introduction

Colorectal cancer is a malignant tumors with high incidence, clinical often take chemotherapy for advanced patients who had lost the opportunity of surgical treatment, but the toxic side effects of chemotherapy may affect the body's immune function[1−3]. Biological therapy is the fourth treatment after the surgery, radiotherapy and chemotherapy and has aroused wide attention clinically[4−6]. Adoptive immunotherapy (ACI) is that autologous or allogeneic immune cells by *in vitro* activation and then infusion in patients to achieve the goal of killing tumor cells in the body[7,8]. At present, the more application was Dendritic cells (DC) combined with cytokine induced killer cells (CIK) [9−11]. Our study aims to observe the effects of DC-CIK on T lymphocyte subsets and cytokines in patients with advanced colorectal cancer.

2. Materials and methods

2.1 Clinical data

Chose 84 cases patients with colorectal cancer who admitted in the Department of oncology, the Fifth People's Hospital of Qinghai Province from August 2014 to August 2015 as the research object. Inclusion criteria: ①Diagnosis by cytology or pathology; ②TNM stage III to IV; ③KPS score ≧70 points; ④Expected survival time ≧6 months; ⑤With normal function of liver, heart, kidney and other organs. Exclusion criteria: ①Combined with other tumor; ②Combined with immune and blood system diseases; ③Combined with serious infection, pneumonia, acute or chronic inflammatory diseases; ④Recent use of immune suppressive agents; ⑤Cannot tolerate chemotherapy. 84 cases patients were randomly divided into two groups, each 42 cases. In the observation group, male 24 cases, female 18 cases; Age from 38 to 70 years old, and the median age was 56 years; 22 cases region in colon and 20 cases in rectum; TNM stage III in 28 cases and Ⅳ in 14 cases. In the control group,

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Fund: Natural Science Foundation Project of Science and Technology Department of Qinghai Province (2014-C-161).

male 26 cases, female 16 cases; Age from 37 to 67 years old, and the median age was 54 years; 21 cases region in colon and 21 cases in rectum; TNM stage III in 30 cases and Ⅳ in 12 cases. There had no significant differences in the general information between the two groups and were comparable. The study was approved by the hospital ethics committee, all subjects were informed and agreed to participate in the research.

2.2 Treatment methods

Both two groups patients were given chemotherapy, using FOLFOX scheme, specifically: the first days, intravenous injection of 85 mg/m^2 oxaliplatin, 200 mg/m^2 Calcium Folinate and 200 mg/m^2 5-fluorouracil. The First and second day, intravenous injection of 600 mg/m^2 5-fluorouracil for 22 h, two weeks a chemotherapy once, two times a course , total 6 courses. On the basis of this, the observation group patients were given supplementary DC-CIK treatment, collected Peripheral blood mononuclear cells (PBMC) from peripheral blood, DC-CIK cells were cultured in the laboratory and were confirmed to be mature DC-CIK cells by Flow cytometry. After passing the test, DC-CIK cells were infusion in patients, once a month, a total 4-6 times.

2.3 Observation indexes

2.3.1 T lymphocyte subsets

3 mL peripheral blood were collected before and after treatment. T lymphocyte subsets CD3$^+$, CD4$^+$, CD8$^+$ and CD4$^+$/CD8$^+$ were detected by flow cytometry (FC 500 flow cytometry, Beckman Coulter, Germany).

2.3.2 Cytokines

3 mL peripheral blood were collected before and after treatment. Centrifugal separation of serum, Th1 cytokines interleukin 2 (IL-2), interferon gamma (IFN-γ) and Th2 cytokines interleukin 6 (IL-6), interleukin 4 (IL-4) were detected by enzyme linked immunosorbent method (ELISA), the kits were purchased from the BD company (United States).

2.4 Statistical treatment

Using SPSS 19.0 software for statistical analysis, the measurement data were expressed by (Mean ± SD), the data were compared by the T test, P<0.05 was considered the difference to be statistically significant.

3. Results

3.1. Comparison the changes of T lymphocyte subsets of the two groups before and after treatment

Before treatment, there had no significant difference in T lymphocyte subsets between the observation and the control group (P>0.05), after treatment, CD3$^+$, CD4$^+$ and CD4$^+$/CD8$^+$ in the observation group were significantly higher than those before treatment, and CD3$^+$, CD4$^+$ and CD4$^+$/CD8$^+$ in the control group were significantly lower than those before treatment, and the differences were statistically significant (P<0.05); CD3$^+$, CD4$^+$ and CD4$^+$/CD8$^+$ in the observation group were significantly higher than those in the control group after treatment (P< 0.05); There had no significant change in CD8$^+$ level between the two groups before and after treatment (P>0.05), see table 1.

3.2 Comparison the changes of Th1 and Th2 cytokines of the two groups before and after treatment

Before treatment, there had no significant difference in Th1 and Th2 cytokines between the observation and the control group (P>0.05), after treatment, Th1 cytokines IFN-γ and IL-2 in the observation group were significantly higher than those before treatment, and Th1 cytokines IFN-γ and IL-2 in the control group were significantly lower than those before treatment, and the differences were statistically significant (P<0.05); Th1 cytokines IFN-γ and IL-2 in the observation group were significantly higher than those in the control group after treatment (P< 0.05); There had no significant changes in Th2 cytokines IL-4 and IL-6 between the two groups before and after treatment (P>0.05), see table 2.

4. Discussions

Epidemiological data showed that the incidence of colorectal cancer had risen to fifth and the mortality rate was sixth in the world[12]. More than 1/4 patients were advanced patients who had lost the chance of surgery, and more than half of the patients with recurrence and metastasis within 5 years after surgical treatment[13]. Therefore colorectal cancer need comprehensive treatment mode. For advanced patients, although a new generation of chemotherapy drugs like oxaliplatin improves the chemotherapy efficiency, but the drugs will cause damage to normal cells when killing tumor cells. In addition, chemotherapy only killing a certain number and proportion of tumor cells, and cannot completely remove the tiny lesions. Studies also found that chemotherapy can cause a transient immune suppression

Table 1

Comparison the changes of T lymphocyte subsets of the two groups before and after treatment.

Group	n	Time	CD3$^+$ (%)	CD4$^+$ (%)	CD8$^+$ (%)	CD4+/CD8$^+$
Observation	42	Before treatment	67.65±5.32	40.23±3.69	27.43±2.91	1.47±0.20
		After treatment	74.42±5.58^{ab}	45.94±3.58^{ab}	29.08±2.66	1.58±0.23^{ab}
Control	42	Before treatment	66.94±5.21	39.68±3.77	27.31±2.88	1.45±0.22
		After treatment	61.18±5.80^{a}	34.68±3.25^{a}	26.86±2.72	1.29±0.21^{a}

Ps: Compared with before treatment, ^aP<0.05; Compared with the control group after treatment, ^bP<0.05.

Table 2

Comparison the changes of Th1 and Th2 cytokines of the two groups before and after treatment (ng/L).

Group	n	Time	Th1		Th2	
			IFN-γ	IL-2	IL-4	IL-6
Observation	42	Before treatment	7.62±1.33	8.09±1.40	2.86±0.56	2.74±0.48
		After treatment	10.56±2.26^{ab}	11.18±2.24^{ab}	2.80±0.49	2.71±0.46
Control	42	Before treatment	7.59±1.26	8.13±1.35	3.01±0.54	2.80±0.50
		After treatment	5.38±0.67^{a}	6.04±1.00^{a}	2.93±0.52	2.79±0.51

Ps: Compared with before treatment, ^aP<0.05; Compared with the control group after treatment, ^bP<0.05.

and effect anti tumor immune function.

Cellular immunity is the main power of anti tumor immunity, and the levels of cytokines and T lymphocyte subsets are the major indexes. T lymphocyte subsets mainly divided into $CD4^+$ and $CD8^+$ according to different functions, both two categories including immune suppressive cells and anti tumor immune cells; $CD3^+$ represents the mature T cells and reflects the immune function[14]. $CD3^+$ and $CD4^+/CD8^+$ keep in balance in normal body, the broken $CD4^+/CD8^+$ balance or the decreased total number of T cells represent the reduced immune function of the body[15]. $CD4^+$ cells can secrete Th0, Th1 and Th2 three different cytokines, Th0 is the immature precursor cells and finally differentiate into Th1 and Th2 cells, Th1 cells mainly secrete IFN-γ and IL-2, which can enhance the immune activity of killer cells and has a positive regulatory immune function[16]; Th2 cells mainly secrete IL-4, IL-6 and has a negative regulatory immune function. Th1/Th2 keep a relative balance in normal people, but Th1 "drift" to Th2 in patients with colorectal cancer, it increase the negative immune regulatory factors, influence the scavenging effect of the immune system on tumor cells[17]. Our study found that in the control group with conventional chemotherapy, $CD3^+$, $CD4^+$, $CD4^+/CD8^+$ and Th1 cytokines IFN-γ, IL-2 were significantly lower compared with those before treatment, and the differences were statistical significance while $CD8^+$ and Th2 cytokines IL-4, IL-6 had no significant differences, which further proved that chemotherapy can kill the normal cells of the body and cause anti tumor cell immune function damage, represent for the decreased positive immune regulatory factor and total number of T cells, and the imbalance of $CD4^+/CD8^+$.

(ACI) is that autologous or allogeneic immune cells by in vitro activation and then infusion in patients to achieve the goal of killing tumor cells in the body. DC and CIK are the two most powerful kinds of antigen presenting cells, DC can induce antigen-specific cytotoxic T lymphocyte reaction and let the effective T cells transfer to tumor site[18]. CIK is a group of heterogeneous cells with a broad spectrum and a high activity of killing tumor, which can through a variety of mechanisms directly or indirectly kill tumor cells[19]. We used DC-CIK treatment for advanced colorectal cancer patients. Our study found that in the observation group with DC-CIK treatment, $CD3^{\pm}$, $CD4^+$, $CD4^+/CD8^+$ and Th1 cytokines IFN-γ, IL-2 were significantly higher compared with those before treatment and the control group after treatment, and the differences were statistical significance. The results showed that DC-CIK treatment could significantly improve the immune function and enhance the anti tumor immune response. DC cells can stimulate the initial activation of T cell proliferation and induce cytotoxic lymphocyte maturation. CIK can secrete large amounts of Th1 cytokines IFN-γ, IL-2 that can kill autologous tumor cells, enhance immune effector cell cytotoxicity. Studies have shown that DC-CIK co culture can play a synergistic role, DC antigen improves and the ability of the immune response are stimulated, the cytotoxic activity of CIK cells and its proliferation in vitro and in vivo is strengthened, which can also promote two cell maturation, when infused into the body of the patient, they can play a synergistic effect of killing tumor[20,21].

References

[1] Yang Wen, Yu Xiaohong, Yang Zhijian. Clinical manifestations of Colorectal cancer on timely diagnosis. *Practical Geriatrics* 2016; **30**(02): 158-161.

[2] Shibutani M, Maeda K, Nagahara H. Prognostic significance of the preoperative ratio of c-reactive protein to albumin in patients with colorectal cancer. *Anticancer Res* 2016; **36**(3): 995-1001.

[3] Huo YR, Huang Y, Liauw W. Prognostic value of carcinoembryonic antigen (cea), afp, ca19-9 and ca125 for patients with colorectal cancer with peritoneal carcinomatosis treated by cytoreductive surgery and intraperitoneal chemotherapy. *Anticancer Res* 2016; **36**(3): 1041-1049.

[4] Wang Haiyan, Xu Chunwei, Wu Yongfang. Molecular pathology analysis of KRAS and BRAF gene mutations in colorectal cancer tissues. *Guizhou Med* 2015; **39**(11): 961-963.

[5] Namdar A, Mirzaei HR, Hafezi M. Low noncytotoxic concentrations of 5-fluorouracil have no adverse effects on maturation and function of bone marrow-derived dendritic cells *in vitro*: a potentially safe adjuvant for dendritic cell-based cancer therapy. *Int Arch Allergy Immunol* 2015; **168**(2): 122-130.

[6] Brackett CM, Kojouharov B, Veith J. Toll-like receptor-5 agonist, entolimod, suppresses metastasis and induces immunity by stimulating an NK-dendritic-$CD8^+$ T-cell axis. *Proc Natl Acad Sci USA* 2016; **113**(7): E874-883.

[7] Wu Hong Mei, Chen Chong. The value of CEA, CA199 and CA125 detection in the diagnosis of colorectal cancer. *J Hainan Med Univ* 2014; **20**(05): 643-645+649.

[8] Son KJ, Choi KR, Lee SJ. Immunogenic cell death induced by ginsenoside rg3: significance in dendritic cell-based anti-tumor immunotherapy. *Immune Netw* 2016; **16**(1): 75-84.

[9] Lee EJ, Lee SJ, Kim JH. Radiation inhibits interleukin-12 production via inhibition of c-rel through the interleukin-6/signal transducer and activator of transcription 3 signaling pathway in dendritic cells. *PLoS One* 2016; **11**(1): e0146463.

[10] Zhang L, Zhu W, Li J. Clinical outcome of immunotherapy with dendritic cell vaccine and cytokine-induced killer cell therapy in hepatobiliary and pancreatic cancer. *Mol Clin Oncol* 2016; **4**(1): 129-133.

[11] Wimmers F, Aarntzen EH, Duiveman-deBoer T. Long-lasting multifunctional CD8 T cell responses in end-stage melanoma patients can be induced by dendritic cell vaccination. *Oncoimmunology* 2015; **5**(1): e1067745.

[12] Wu X, He X, Li S. Long Non-coding RNA ucoo2kmd.1 regulates CD44-Dependent cell growth by competing for miR-211-3p in colorectal cancer. *PLoS One* 2016; **11**(3): e0151287.

[13] Militello LG, Saleem JJ, Borders MR. Designing colorectal cancer screening decision support: a cognitive engineering enterprise. *J Cogn Eng Decis Mak* 2016; **10**(1): 74-90.

[14] Demoulin SA, Somja J, Duray A. Cervical (pre)neoplastic microenvironment promotes the emergence of tolerogenic dendritic cells via RANKL secretion. *Oncoimmunology* 2015; **4**(6): e1008334.

[15] Søndergaard JN, Poghosyan S, Hontelez S. DC-SCRIPT regulates IL-10 production in human dendritic cells by modulating nf-κb p65 activation. J Immunol 2015; 195(4): 1498-1505.

[16] Zheng C, Yu G, Wang H. Meta-analysis of chemotherapy and dendritic cells with cytokine-induced killer cells in the treatment of non-small-cell lung cancer. *Int J Clin Exp Med* 2015; **8**(8): 14527-14537.

[17] Song QK, Ren J, Zhou XN. The prognostic value of peripheral $CD4^+CD25^+$ T lymphocytes among early stage and triple negative breast cancer patients receiving dendritic cells-cytokine induced killer cells infusion. *Oncotarget* 2015; **6**(38): 41350-41359.

[18] Lind EF, Millar DG, Dissanayake D. miR-155 upregulation in dendritic cells is sufficient to break tolerance in vivo by negatively regulating SHIP1. *J Immunol* 2015; **195**(10): 4632-4640.

[19] Radice E, Bellone G, Miranda V. Enhancement of the immunostimulatory functions of *ex vivo*-generated dendritic cells from early-stage colon cancer patients by consecutive exposure to low doses of sequential-kinetic-activated IL-4 and IL-12. A preliminary study. *Transl Oncol* 2015; **8**(4): 327-338.

[20] Dong M, Wang X, Liu J. Rapamycin combined with immature dendritic cells attenuates obliterative bronchiolitis in trachea allograft rats by regulating the balance of regulatory and effector T cells. *Int Arch Allergy Immunol* 2015; **167**(3): 177-185.

[21] Thomann S, Boscheinen JB, Vogel K. Combined cytotoxic activity of an infectious, but non-replicative herpes simplex virus type 1 and plasmacytoid dendritic cells against tumour cells. *Immunology* 2015; **146**(2): 327-238.