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ORIGINAL ARTICLE

Combined Treatment with Autologous CIK Cells, Radiotherapy and Chemotherapy in Advanced Cervical Cancer

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Abstract

To investigate the clinical efficacy of autologous cytokine induced killer (CIK) cells transfusion combined with radiochemotherapy in the treatment of advanced cervical cancer. A total of 89 hospitalized patients with advanced cervical cancer were admitted and divided into the treatment group (44 cases, autologous CIK cells transfusion combined with radiochemotherapy) and the control group (45 cases, radiochemotherapy) by a randomized non-blind method. Comparisons of therapeutic efficacies, immune functions, life qualities and survival rates were analyzed between the two groups. The short-term therapeutic efficacy of the treatment group was significantly higher than that of the control group. There was no significant difference in 1, 2 and 3 year survival rates between the two groups. Compared with pre-treatment, levels of $CD3^+$, $CD4^+/CD8^+$ in peripheral blood were increased in the CIK group, which were reduced in the control group. In the CIK group,only the feeling was depressed on the 25th day post-treatment (T25) compared with the day before treatment (B1). However in the control group, the function of body, role, social and holistic health was obvious disordered on day T25 compared with day B1. On day T25, there were significant differences in function of body, social and holistic health between two groups. Autologous CIK cells transfusion combined with radiochemotherapy shows better short-term efficacy than radiochemotherapy alone in the treatment of advanced cervical cancer, which obviously improves immune function and life quality of patients with low side effects.

Keywords Autologous CIK cell · Advanced cervical cancer · Radiochemotherapy

Introduction

Cervical cancer is one of the most common gynecological malignancy, and it is the fourth leading cause of cancer-related deaths among women around the world for several decades [1]. Cervical cancer accounts for 9% of the total new cancer cases and 8% of the total cancer deaths among females in 2008 [2]. Generally, a majority of these cases and deaths occur in developing countries [3]. Females often have a higher prevalence rate under the conditions of early marriage, childbearing, human papillomavirus virus (HPV) infection, and most of them are first diagnosed with middle or late stage due to the rapid development of the disease [4]. Clinically, several therapeutic approaches are available for cervical cancer, including surgery, radiotherapy and chemotherapy, but the outcome remains unsatisfied due to heavy side effects and poor therapeutic efficacy [5]. Thus, there is still a need for understanding the molecular mechanisms involved in cervical cancer that may lead to new diagnostic and therapeutic targets.

Nowadays, cytokine induced killer (CIK) cells have been widely applicated in cancers. CIK cells present anti-tumor activity against a variety of malignancies in preclinical models and prove to be safe and effective in clinical trials [6, 7]. Adoptive immunotherapy for the transport of immunocompetent cells in vivo is becoming a new treatment apart from surgery, radiotherapy and chemotherapy [8]. Recent evidence has suggested that CIK cells play a vital role in the regulation of cervical cancer. For example, CIK cells have a stronger suppressive effect on tumor growth in BALB/c nude mice bearing cervical cancer than lymphokine activated killer cells. The tumor size in the experiment group is smaller than that in the control group after CIK treatment [9]. Kim et al. has

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reported that CIK cells destroy 56% of KB-3-1 human cervical cancer cells at an effector-target cell ratio of 100:1 in vitro, and CIK cells at doses of 3 and 10 million cells per mouse inhibit 34% and 57% of KB-3-1 tumor growth in nude mouse xenograft assays respectively [10]. In the current study, the therapeutic efficacy of autologous CIK cells transfusion combined with radiochemotherapy in forty-four patients with advanced cervical cancer was observed and followed up from October 2010 to July 2015.

Materials and Methods

Patients

Total 89 cases of cervical cancer patients were observed in this study, including squamous cell carcinoma in 77 cases, adenocarcinoma in 8 cases, and other (small cell carcinoma and adenosquamous carcinoma) 4 cases. Clinical staging was performed according to the International Federation of Gynecology and Obstetrics (FIGO) criteria: Stage II A-II B 34 cases, Stage III A-III B 46 cases, Stage IV 9 cases. The patients were 36~80 years of age with the median age of 54. Among them, 40.45% (36 patients) underwent surgery or chemoradiotherapy six months before participation. Inclusion criteria: patients were diagnosed with cervical cancer by imaging, pathology and cytology. Karnofsky (KPS) score was more than 70 points. Liver function, kidney function and blood routine were normal without serious heart, liver or kidney diseases. The predicted survival time was more than 3 months. All of them were evaluated to fit radiotherapy, chemotherapy and cell therapy. Patients were divided into the treatment group (44 cases) or the control group (45 cases) by a randomized non-blind method. The clinicopathological patterns of the tumors on both subject groups were shown in Table 1. Informed consent was obtained from all individual participants included in the study. This study was approved by the Research Ethics Committee of Kaifeng Central Hospital. The patients were followed up by telephone. Progression-free survival time was observed after disease progresses, and none of the patients lost follow-up. There was no significant difference between the two groups in gender, age, pathological type, clinical stage, the number of treatment lines or the average number of chemotherapy cycles.

Reagents and Instruments

Interferon (INF)- γ was purchased from Shanghai Kai Mao Biological Medicine Co., Ltd. Interleukin (IL)- 1α was purchased from Shanghai Puxin Biotechnology Co., Ltd. IL-2 was purchased from Beijing Shuanglu Pharmaceutical Co., Ltd. CD3 monoclonal antibody was purchased from Miltenyi Biotec (Germany). Human lymphocyteseparation solution was purchased from Hao Yang Biological products science and technology Co., Ltd. (Tianjin, China). RPMI medium was bought from GIBCO. Human serum albumin (HSA) was obtained from Hua Lan Biological Engineering Co., Ltd. (Suzhou, China). The dye trypan blue was purchased from Sigma. Flow cytometry detection antibodies CD3-FITC, CD8-PE and CD56-PE were purchased from BD (Shanghai, China). Centrifuge tubes (50 mL) were purchased from Corning. Cell culture flasks (175 cm) were bought from Nunc. Cell culture bags (640 cm^2) were purchased from Takara. Type SW-CJ-2D super-clean work table was purchased from Suzhou purification equipment Co., Ltd. (Suzhou, China). Type 311 CO_2 cell incubator and ST-40R centrifuge were purchased from Thermo Scientific company. Type ECLIPSE TS100-F inverted biological microscope was purchased from Nikon (Shanghai, China). Type BC-3000 cell counter was purchased from MaiRui medical electronic Co., Ltd. (Shenzhen, China). Type BDFACSCalibur flow cytometry was purchased from BD (Shanghai, China).

Table 1 Clinicopathological patterns of the tumors on both subject groups (n (%))

Groups	Pathological type	Clinical Stage			Total
		IIA-IIB	IIIA-IIIB	IV	
Treatment	Squamous cell carcinoma	16	18	4	38
	Adenocarcinoma	1	3	0	4
	Other	0	2	0	2
Control	Squamous cell carcinoma	15	19	5	39
	Adenocarcinoma	2	2	0	4
	Other	0	2	0	2
Total		34 (38.2)	46 (51.7)	9 (10.1)	89

Therapeutic Method

Preparation and Transfusion of Autologous CIK Cells

Mononuclear cells were isolated from autologous peripheral blood using a Ficoll method. Then they were washed with RPMI 1640 three times and suspended in RPMI 1640 complete medium at a cell density of 2×10^6 /mL. The procedure of autologous CIK cells culture and transfusion were as follows: transfusion 1 time per day with total 4 days as a course. Peripheral blood was collected on the day before the first chemotherapy (4 weeks post-radiotherapy) in the CIK treatment group to isolate and culture CIK cells. After 14 days of culture, CIK cells transfusion was performed and the second chemotherapy was carried out one week after the course. Second collection of peripheral blood was performed on the day before the second chemotherapy. Chemotherapy alternates with CIK cells transfusion for totally 4 ~ 6 courses in CIK treatment group.

Radiotherapy and Chemotherapy Protocols

The chemotherapy regimen used in the two groups was paclitaxel or gemcitabine combined with cisplatin, every 28 d for one cycle. Conformal radiotherapy combined with intracavitary brachytherapy was used in the radiotherapy process. The first dose of patients with radical radiotherapy was whole pelvic irradiation 30 ~ 36 Gy, four fields box of types 20 Gy, combined with intracavitary irradiation 30 ~ 38 Gy, with a dose of 70~76 Gy at point A and 50 ~ 56 Gy at point B. The radiation dose in patients with pelvic recurrence was GTV 60 ~ 66 Gy. Patients were treated simultaneously with radiotherapy and combination chemotherapy.

Outcome Measures

Comparisons of tumor size, immune functions, survival rates and qualities of life of the patients in two groups were analyzed. The standard of WHO judging solid tumors was used to evaluate tumor size, which was based on CT/MR scans 1 month before and after the treatment. Results were shown as complete remission (CR), partial remission (PR), stable disease (SD) and progressive disease (PD). Objective response rate (ORR) equalled CR plus PR, and disease control rate (DCR) was in total of CR, PR and SD. Immune functions: levels of $CD3^+$, $CD4^+$ and $CD8^+$ were examined one day before the treatment (B1), the fourteenth day of chemotherapy (T14) and the twenty-fifth day of chemotherapy (T25), along with $CD4^+$ /$CD8^+$ ratio. Survival rates: the 1, 2, and 3 year survival rates of the two groups were calculated. Quality of life: KPS score was used to assess the quality of life before and after treatment in the two groups, and increased KPS score was considered to be improved life quality. A questionnaire survey was also conducted using the Chinese version of the European cancer research and treatment organization (EORTC) QLQ-C30 (V3.0) on day B1, T14 and T25.

Statistical Analysis

SPSS 22 was used to analyse the data in the study. χ^2 test was used for group comparison, and measurement data were expressed as means ± standard deviation (SD). Paired t test was used to analyse data before and after treatment, and the independent sample t test was used to compare between the groups. $P < 0.05$ or $P < 0.01$ was considered statistically significant.

Results

Short-Term Therapeutic Efficacy

The effective rate 1 month post-treatment of the CIK treatment group was 88.64% (39/44), while in the control group it was 68.89% (31/45). The difference between the two groups was statistically significant ($P < 0.05$, Table 2).

Immune Function

In the CIK treatment group, the levels of T lymphocyte subsets $CD3^+$ and $CD4^+/CD8^+$ ratio were significantly increased on day T25 compared with day T14 ($P < 0.05$ or $P < 0.01$). The levels of $CD3^+$, $CD4^+$ and $CD4^+/CD8^+$ ratio were slightly decreased on day T14 compared with day B1 ($P > 0.05$). In the control group, the levels of $CD3^+$, $CD4^+$ and $CD4^+/CD8^+$ ratio were increased, while $CD8^+$ level was decreased on day T25 compared with day T14. However, no significant differences were observed among different time-points ($P > 0.05$, Table 3).

Table 2 Short-term therapeutic efficacies of the patients in two groups (n)

Groups	N	CR	PR	SD	PD	ORR (%)
Treatment	44	22	17	3	2	88.64*
Control	45	15	16	8	6	68.89

* $P < 0.05$ vs control group

Table 3 Levels of immunological markers in the patients of two groups at various time-points (%)

Groups	N	Time-point	$CD3^+$	$CD4^+$	$CD8^+$	$CD4^+/CD8^+$
Treatment	44	B1	68.2 ± 7.0	40.5 ± 7.1	35.5 ± 6.9	1.1 ± 0.3
		T14	60.9 ± 10.3	27.9 ± 8.0	26.8 ± 8.9	1.1 ± 1.0
		T25	78.0 ± 4.9**	44.8 ± 5.3	27.5 ± 1.3	1.6 ± 1.0*
Control	45	B1	55.9 ± 7.1	24.8 ± 6.1	25.0 ± 5.9	1.0 ± 0.5
		T14	55.4 ± 9.9	24.0 ± 8.0	25.9 ± 7.1	1.0 ± 0.6
		T25	57.9 ± 7.2	25.6 ± 5.1	23.9 ± 5.0	1.0 ± 0.7

* $P < 0.05$, ** $P < 0.01$ vs treatment group T14

Life Quality

KPS Score

Before starting the treatment, there was no significant difference between the CIK group and the control group. After the treatment, the KPS score of the CIK group was 0 ~ 20 points higher before treatment, which has significant difference compared with the control group ($P < 0.05$). In addition, there was significant difference of KPS score both in the CIK and the control group after treatment ($P < 0.01$). These data revealed that the quality of life was improved in both groups after treatment, and the improvement in the autologous CIK cells treatment group was more obvious (Table 4).

Patient Functional Dimension and General Health

Patient functional dimension and general health scores in two groups were decreased from day B1 to day T14, but were raised from day T14 to day T25. In the CIK treatment group,only the feeling was depressed on day T25 compared with day B1 ($P < 0.05$). However in the control group, the function of body, role, social and holistic health was obvious disordered on day T25 compared with day B1 ($P < 0.05$). On day T25, there were significant differences in function of body, social and holistic health between two groups ($P < 0.05$, Table 5).

Table 4 Karnofsky score of the patients at pre- and post-treatment (score)

Groups	N	Pre-treatment	Post-treatment	*T* value	*P* value
Treatment	44	73.38 ± 9.01	77.67 ± 8.03	−3.843	0.001
Control	45	70.50 ± 6.82	74.81 ± 6.10	−5.682	0.008
T value		1.039	2.215		
P value		0.312	0.031		

Survival Rates

All patients were followed up by telephone review. The follow-up time was 10 ~ 38 months and the follow-up rate was 100%. The 1, 2 and 3 year survival rates in the treatment group (93.18%, 77.27% and 47.73%) were higher than those in the control group (88.88%, 68.89% and 42.22%), although no significant differences were observed ($P > 0.05$).

Adverse Reactions

At the end of CIK cells transfusion, blood routine, liver and kidney function results of all the patients were not significantly changed. The most common adverse reaction was transient hypothermia (15 patients, body temperature ≤ 38. 5 °C) after CIK cells transfusion. It was turned to normal within 24 h without high fever recurrence.

Discussion

At present, the treatment of advanced cervical cancer is the comprehensive treatment based on radiotherapy. Cisplatin based concurrent chemoradiotherapy has been standard regimen in recent years [11]. Although great progress has been made clinically, there are still many drawbacks with disappointing outcomes. Recently, with the in-depth study of tumor immune escape mechanism as well as the rapid development of immune molecular biology, biological treatment of malignant tumors is starching more attention [12]. As the fourth generation of treatment for various cancers, biotherapy has been widely recognized and applied in cancer treatment. CIK cells are heterogeneous ex vivo-expanded T lymphocytes with mixed T-NK phenotype and endowed with a wide major histocompatibility complex (MHC)-unrestricted anti-tumor activity. CIK cells can be expanded from peripheral blood mononuclear cells (PBMC) cultured with the timed addition of IFN-γ, Ab anti-CD3 and IL2 [13], and they are characterized of high proliferation rate and anti-tumor activity, wide

Table 5 Evaluation of functional dimension and overall health status of the patients in two groups (score)

Groups	Time-point	Body	Role	Feelings	Cognition	Social	Holisitic health
Treatment	B1	70.02 ± 11.22	68.32 ± 17.35	68.95 ± 11.72	59.48 ± 15.83	50.75 ± 16.97	68.61 ± 17.56
	T14	65.24 ± 11.49	61.34 ± 18.67	61.87 ± 11.55	56.93 ± 14.68	44.62 ± 16.57	58.04 ± 18.41
	T25	69.53 ± 11.61	65.83 ± 13.50	61.14 ± 14.15*	59.50 ± 14.80	49.62 ± 11.59	65.31 ± 13.20
Control	B1	70.23 ± 11.43	68.14 ± 12.50	64.61 ± 14.55	63.15 ± 15.40	51.63 ± 21.45	70.34 ± 15.86
	T14	51.54 ± 12.60	48.51 ± 19.65	61.87 ± 11.82	57.66 ± 16.84	41.13 ± 16.89	51.50 ± 18.70
	T25	59.50 ± 20.55$^{\&\#}$	56.67 ± 18.80$^{\&}$	65.40 ± 14.69	59.88 ± 19.11	42.88 ± 13.00$^{\&\#}$	58.82 ± 17.22$^{\&\#}$

* $P < 0.05$ vs treatment group B1; $^{\&}$ $P < 0.05$ vs control group B1; $^{\#}$ $P < 0.05$ vs treatment group T25

tumor spectrum and low adverse reactions. In the absence of damage to the immune system, CIK cells can not only directly kill tumor cells but also strengthen body immune function. Additionally, CIK cells transfusion has been widely applicated among tumor patients, especially in advanced cancer patients without surgery, radiotherapy, or chemotherapy indications [14]. Therefore, immunotherapy using CIK cells may become a novel treatment strategy for cancer patients. Generally, body immune function can be restored after 2 ~ 4 weeks of radiotherapy and chemotherapy. At this point, transfusion of autologous CIK cells can improve the remission rate, which play an important role in the removal of minimal residual lesions [15].

CIK cells are usually transfused intravenously in adoptive cellular immunotherapy, and the transfusion of CIK cells demonstrates different anti-tumor effect among diverse tumors. For example, co-cultured CIK cells and dendritic cells (DC), known as DC-CIK cells, combined with chemoradiation therapy is effective and reliable in the treatment of patients with middle-advanced non-small cell lung cancer [16]. It has been reported that as the adjuvant treatment of stage III gastric cancer, CIK cells transfusion could improve the immune function of patients, and prolong overall survival time as well as disease-free survival time [17]. Autologous DC-CIK cells transfusion may improve immune response of patients with metastatic renal cell carcinoma, which has an excellent therapeutic efficacy [18]. Similarly, in postoperative patients with cervical cancer, treatment with DC-CIK cells combined with cisplatin chemotherapy could significantly improve the immune function, reduce the recurrence rate and prolong survival time [19].

In the current study, autologous CIK cells transfusion was conducted with chemoradiotherapy in patients of advanced cervical cancer. Results showed that tumor size was significantly decreased and short-term therapeutic efficacy was obviously increased compared with the control group. It also improved the 1, 2, 3 year survival rates although the difference between the two groups was not significant. Speaking of the quality of life, KPS score of CIK cells treatment group, along with function of body, social and holistic health was significantly improved than those of control group. Immune function was obviously restored one week after CIK cells transfusion; while it continued to fall down in the control group at the same time-point, although the difference was not significant. Among CIK cells transfusion patients, only a small amount appeared transient hypothermia. Taken together, these data showed that autologous CIK cells transfusion can improve life quality of patients with low adverse reactions. However, the number of cases in this research is limited, so we should continue to expand cases to further investigate a novel treatment strategy for patients with advanced cervical cancer.

In conclusion, our results indicate that autologous CIK cells transfusion combined with chemoradiation therapy was confirmed to be an ideal treatment of advanced cervical cancer with the advantage of higher short-term therapeutic efficacy, improve of immune function and life quality with low side effects.

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Compliance with Ethical Standards

Conflict of Interest The authors report no potential conflict of interest.

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