GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Efficacy of dendritic cell-cytokine-induced killer immunotherapy plus intensity-modulated radiation therapy in treating elderly patients with esophageal carcinoma

L. Yan, M. Wu, N. Ba, L.J. Wang, H.Q. Zhang, G.Y. Shi, Z.S. Zhang and X.J. Wang

Cancer Biotherapy Center, The Fifth Affiliated Hospital of Zhengzhou University, Zhengzhou, China

Corresponding author: X.J. Wang
Email: wangxinjun14@sina.com

Genet. Mol. Res. 14 (1): 898-905 (2015)
Received May 15, 2014
Accepted October 22, 2014
Published February 2, 2015
DOI http://dx.doi.org/10.4238/2015.February.2.13

ABSTRACT. We investigated the clinical efficacy of adoptive cytokine-induced killer (CIK) cell and dendritic cell (DC) therapy plus intensity-modulated radiation therapy (IMRT) for treating elderly patients with esophageal carcinoma (EC). In total, 68 elderly patients with EC were randomized to receive IMRT plus DC-CIK immunotherapy (study group, N = 34) or IMRT only (control group, N = 34). Clinical efficacy, immune function, toxicity and side effects, and life quality were evaluated after treatment. The efficacy rate was significantly higher in the study group than in the control group. Remarkable increases were noted for quality of life and immune function in the study group relative to the control group. Regarding toxicity and side effects, compared with the control group, the study group displayed a higher fever rate, a lower incidence rate of bone marrow suppression, and a similar rate of digestive tract reactions. DC-CIK immunotherapy plus IMRT exhibited

better short-term efficacy than IMRT alone in elderly patients with EC. The therapy could improve patients' quality of life and immune function, decrease bone marrow suppression, and lengthen survival time.

Key words: Immunotherapy; Radiotherapy; Esophageal carcinoma; Aged population

INTRODUCTION

Esophageal carcinoma (EC), a common gastrointestinal malignancy, has been identified as a major threat to public health in China. To the best of our knowledge, the incidence of EC is comparatively lower than that of other malignant tumors worldwide. However, a higher incidence of EC was noticed in Henan Province in northern China, with a 5-year overall survival rate of less than 20% (Wang et al., 2002; Ma et al., 2009). Furthermore, the incidence is increasing annually.

Among elderly patients, moderately advanced or advanced EC is commonly identified for various reasons, including decreased self-care capacity and economic conditions. Unfortunately, one or more chronic diseases such as poor organ tolerance have been reported in these populations (Faiz et al., 2012). At the same time, surgery and chemotherapy are not preferred for these patients because of the complex structure of the esophagus, lymph node metastasis, and other factors. Recently, cytokine-induced killer (CIK) cell and dendritic cell (DC) immunotherapy has been used as a local treatment for malignant carcinoma, including prostate, pancreatic, and lung cancers (Shi et al., 2012; Cui et al., 2013; Yuan et al., 2013). CIK-DK immunotherapy works on the premise that autologous DCs can activate CIK cells to enhance the tumor-killing effects, based on which the immune system can distinguish cancer cells from the transfused normal cells cultured *ex vivo*. To our knowledge, most prior studies investigated the effects of DC-CIK immunotherapy in treating lung carcinoma, especially non-small-cell lung carcinoma (NSCLC). However, few studies investigated the effects of DC-CIK immunotherapy in patients with EC.

Intensity-modulated radiation therapy (IMRT) has been commonly used in radiotherapy in recent years. Compared with conventional techniques, IMRT displayed the advantages of more precisely delivering radiation while limiting the dose to the surrounding normal tissues (Son et al., 2012). Indeed, this therapy has limitations, including diminished efficiency in treating EC. We hypothesized that the inherent limitation of IMRT may be overcome in combination with DC-CIK immunotherapy. Thus, in our study, we applied IMRT combined with DC-CIK immunotherapy to treat elderly patients with EC.

MATERIAL AND METHODS

Agents

GT-T551 serum-free culture medium was purchased from Takara (Dalian) Co., Ltd. (Dalian, China). Interleukin (IL)-1 and IL-4 were purchased from Shanghai Claison Bio-tech Co., Ltd. (Shanghai, China). Ficoll hydroxypropyl methylcellulose was purchased from GE (Beijing) Co., Ltd. (Beijing, China). Interferon (IFN)-γ was provided by Shanghai Kelong Co., Ltd. (Shanghai, China). IL-2 was purchased from SL Pharmaceutical Co., Ltd. (Shanghai, China).

Patients

A total of 68 patients (45 male and 23 female) with squamous cell carcinoma of the-esophagus were included in this study. The patients were aged 65-79 years, with a median age of 70 years. Among these patients, the numbers of patients diagnosed with stage I, II, III, and IV EC were 3, 12, 29, and 24, respectively, according to the tumor-node-metastasis system.

The inclusion criteria were as follows: 1) pathologically and cytologically confirmed EC; 2) age ≥ 65 years and a Karnofsky score ≥ 70; 3) no history of antitumor therapy prior to this study; 4) no anomalies in routine blood, hepatic function, and renal function testing; 5) no severe heart, hepatic, or kidney disease; and 6) measurable tumor lesions. The exclusion criteria were as follows: 1) intracranial metastasis of tumor cells; 2) severe functional defects of the heart, liver, or kidneys; 3) concomitant malignant tumors; and 4) an inability to finish this study because of mental illness. For the patients concurrent with chronic diseases, such as hypertension, diabetes mellitus, coronary heart disease, chronic obstructive pulmonary disease, and rheumatism, symptomatic therapy was administered to maintain a suitable health condition to guarantee the progression of the study. All patients provided written informed consent. This study was approved by the Ethics Committee of the Fifth Affiliated Hospital of Zhengzhou University.

Treatment

The patients were randomly divided into the study group (N = 34), which was administered IMRT combined with DC-CIK immunotherapy, and thecontrol group, which was given IMRT only (N = 34). Randomization was performed using random number generation methods.

For IMRT, extracorporeal irradiation was administered using a medical linear accelerator with 6MV X-rays. Spiral computed tomography (CT) was then performed to access the movement of the target. The gross tumor volume was defined as the gross disease identified in the CT, including the primary esophageal lesion and metastatic lymph nodes. The clinical target volume (CTV) included subclinical lesions (3 cm from the superior and inferior margins of EC) and local lymph nodes. The planning tumor volume was defined as an expansion of the CTV by a 5-8-mm margin, and it was covered by the 95% isodose. Dose fractionation irradiation was used during the procedure for 6-7 weeks with a total dose of 60-66Gy (5 times/week, 2Gy/session, once/day).

DC-CIK immunotherapy

The patients in the study group completed 5 adoptive DC cell-CIK cell transfer sessions. The DC and CIK cells were harvested according to Good Manufacturing Practice guidelines as previously described (Li et al., 2009; Yang et al., 2013). Briefly, 2 days before IMRT, peripheral blood mononuclear cells were suspended in a medium containing IL-2, CD3, and IFN-γ to culture CIK cells. After continuous passage, the cells that adhered to the flasks were removed with a cell spatula and centrifugation at 3500 rpm at 25°C. Then, the cells were suspended in DC-CIK medium containing IL-2 and a monoclonal antibody against CD3 and cultured for 48 hours. Finally, the DC and CIK cells were harvested and suspended in saline for intravenous injection. For quality control, the dyeexclusion test was performed to test the viability of the cells. Meanwhile, possible contamination with bacteria, fungi, and endotoxins

was tested by a qualified technician.

For the patients in the study group, IMRT was administered on day 3 and terminated 2 days before transfusion of the DC and CIK cells. For each session, 200 mL DC and CIK cells containing $1x10^9$ CIK cells and $1x10^7$ DC cells were transfused each dayfor 5 days. Approximately 30 minutes prior to cell transfusion, diphenhydramine (20 mg) was injected intramuscularly.

Evaluation of clinical safety and side effects

After treatment, routine blood, blood glucose, and blood pressure tests and electrocardiography were performed once a week. Meanwhile, side effects including hepatic and renal dysfunction, fever, allergy, and bone marrow depression were assessed. Bone marrow suppression manifested as decreases in white blood cell, neutrophil, and platelet counts and hemoglobin levels. Toxicity and esophageal reactions were categorized into 5 levels (level 0-5) according to the radiation injury evaluation standard proposed by the Radiation Therapy Oncology Group.

Determination of immune indices

To determine the alternation of immune indices, subgroups of peripheral blood T lymphocytes were tested before and after treatment, including $CD3^+$, $CD3^+CD4^+$, $CD3^+CD8^+$, and NK ($CD3^-CD56^+$).

Evaluation of clinical efficacy

Clinical efficacy was divided into 4 grades, namely complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD), according to Response Evaluation Criteria in Solid Tumors. Imaging was performed at 1, 2, and 3 months after treatment, with the 3-month imaging serving as the most recent response evaluation criteria. Imaging evaluations were conducted every 3 months after the treatment.

Follow-up

Periodic follow-up was performed via telephone communication from October 2012 to October 2013. The mean follow-up duration was 6.7 months.

Statistical analysis

All data are reported as means ± standard deviation. Treatment efficacy was analyzed by the nonparametric rank sum test. The chi-squared test was used to analyze life quality between the study and control groups. Student *t*-test was used for intergroup comparisons.

RESULTS

Patient information

The patient information for both groups is presented in Table 1. No statistical differences were noted between the groups before the study ($P > 0.05$).

Table 1. Clinical data of the patients.

Characters	Study group	Control group
Gender		
Male	22	23
Female	12	11
Age (years)	70.46 ± 2.91	71.55 ± 2.23
Tumor stage		
Stage I	2	1
Stage II	6	7
Stage III	15	14
Stage IV	11	12
Pulmonary metastasis	4	3
Hepatic metastasis	2	2
Other metastasis	5	8
Concurrent chronic diseases		
Diabetes mellitus	8	6
Hypertension	11	13
Chronic obstructive pulmonary disease	10	9
Other chronic diseases	4	3

Follow-up evaluation

No patient was lost during the follow-up period. The median follow-up period was 15 months (range, 4-23 months) in the study group, and the median time to progression (TTP) was 13.6 months. In the control group, the median follow-up period was 15 months (range, 3-22 months), and the median TTP was 12.7 months.

Comparison of T cell subgroups

The prevalence of T cell subgroups including $CD3^+$, $CD3^+CD4^+$, $CD3^+CD8^+$, and NK ($CD3^-CD56^+$) in peripheral blood is an important index reflecting the immune status of cells. Table 2 summarizes the prevalence of T cell subgroups after treatment. $CD3^+$, $CD3^+CD4^+$, and $CD3^+CD8^+$cell numbers were obviously increased in the study group compared with the baseline levels ($P < 0.05$). Compared with the control group, the numbers of $CD3^+CD4^+$, $CD3^+CD8^+$, and NK ($CD3^-CD56$) positive cells were superior after treatment ($P < 0.05$).

Table 2. Comparison of T cell subgroups before and after treatment.

Group	$CD3^+$		$CD3^+CD4^+$		$CD3^+CD8^+$		NK	
	Baseline level	Post-treatment	Baseline level	Post-treatment	Baseline level	Post-treatment	Baseline level	Post-treatment
Study group	54.24 ± 7.31	69.17 ± 6.11*	31.92 ± 5.96	38.84 ± 4.19*,†	27.56 ± 5.45	36.12 ± 4.31*,†	10.32 ± 3.69	12.14 ± 5.19†
Control group	55.43 ± 8.12	54.69 ± 7.64	31.17 ± 5.81	30.04 ± 6.18	27.47 ± 6.12	26.33 ± 5.59	9.87 ± 7.23	9.01 ± 6.25

*$P < 0.05$, compared with baseline level; †$P < 0.05$, compared with control group.

Comparison of short-term treatment efficacy

Three months after treatment, a response evaluation of the treatment plan was performed according to the number of lesions in the radiation field, which revealed that CRs occurred in both groups. For the study group, CRs and PRs were noted in 4 (11.8%) and 10 patients (29.4%), respectively. In addition, SD was observed in 15 patients (44.1%), whereas 5 patients (14.7%) displayed PD. In total, the objective response rate was 41.2%, and the disease

control rate was 85.3%. In the control group, CRs and PRs were observed in 3 (8.8%) and 7 patients (20.6%), respectively. In addition, SD was noted in 10 patients (29.4%), whereas 14 patients (41.1%) exhibited PD. The objective response rate was 29.4%, and the disease control rate was 61.7%. Significant differences in treatment efficacy were observed between the groups ($P < 0.05$).

In this study, metastasis and the number of lesions outside the radiation field were also evaluated. In the study group, progression and metastasis of the tumor lesion were identified in 9 patients (26.5%) using CT. In the control group, 12 patients exhibited progression and metastasis of the tumor lesion using the same technique. No statistical difference was noticed between the groups ($P > 0.05$).

Side effects

In the study group, the major side effects included shiver and severe fever (N = 3), overexcitation (N = 12), and insomnia due to overexcitation (N = 4). These symptoms were partially or completed relieved after symptomatic treatment. In the control group, the side effects included fever (N = 1) as well as overexcitation and/or insomnia (N = 5). Compared with the control group, only the incidence of overexcitation was significantly different in the study group ($P < 0.05$).

After IMRT and DC-CIK immunotherapy, mild (N = 2) or moderate (N = 2) bone marrow suppression was noted in the study group. Meanwhile, in the control group, severe bone marrow suppression was noted in a number of patients, including degree I (N = 9) and II (N = 6) suppression. Compared with that in the control group, the incidence of bone marrow suppression was remarkably decreased in the study group ($P < 0.05$).

Regarding digestive tract reactions, grade I and/or II reactions were commonly observed in the study and control groups. In the study group, the numbers of patients with grade I and II digestive tract reactions were 16 and 3, respectively, compared to 20 and 5 patients respectively, in the control group. No statistical difference was revealed by intergroup comparison.

Tracheitis is a common complication after radioactive therapy. In our study, the numbers of patients with grade I and II tracheitis were 9 and 2, respectively. In the control group, 9 patients displayed grade I tracheitis after treatment. No allergy, severe liver/renal functional lesions, or complicated systemic disease aggravation was identified in the 2 groups.

Clinical safety

The mortality rates were 44.1% and 58.8% in the control and study groups, respectively. Cause-of-death analysis indicated that EC relapsed in 5 patients within the radiation field in the study group. Additionally, progression and metastasis outside the radiation field were observed in 8 patients. Further, EC relapse within the radiation field combined with progression and metastasis outside the radiation field were observed in 2 patients in the study group. In the control group, relapse within the radiation field was identified in 6 patients, whereas progression plus metastasis was noted in 10 patients. Moreover, relapse within the radiation field combined with progression and metastasis outside the radiation field was observed in 4 patients in the control group.

Comparison of life quality

In the study group, 21 patients reported improvements of life quality, and 10 patients exhibited stable health conditions. Nevertheless, deterioration was noticed in 2 patients. On the contrary, 14 patients in the control group reported improvements of life quality, and 8 patients displayed stable health conditions. However, deterioration was noted in 12 patients. Compared with the control group, the overall quality of life was improved in the study group ($P < 0.05$).

DISCUSSION

A high incidence of EC was reported in Henan Province in China. As elderly patients with EC typically present with one or more chronic diseases, radical surgery and/or chemotherapy are not preferred in clinical practice. Therefore, radiotherapy is commonly used for these populations. Nevertheless, its clinical efficiency has been undermined by the existence of radiotherapy-resistant cells, which comprise approximately 10-50% of the solid tumor on average. To solve this problem, enhanced-dosage radiotherapy has been proposed. Nevertheless, for the elderly population with chronic organ failure, the aforementioned regimen is not recommended, as it could present a great threat to the safety of the patients.

Three-dimensional (3D) conformal modulating radiotherapy has been commonly used in the treatment of carcinoma, as the shape of the radiotherapy field and irradiated dose in the 3D space were consistent with the actual shape of the tumor. This method increased the dose of the radiation source in the target tumor with less toxicity against the normal tissues. Unfortunately, its efficacy in treating EC is still comparatively low, with a higher incidence of relapse and a short survival time. Furthermore, lower tolerance to radiotherapy has been frequently reported in elderly patients. To solve this problem, we proposed IMRT combined with DC-CIK immunotherapy for the treatment of elderly patients with EC. We hoped to improve the treatment efficacy in this population through 2 aspects. i) The CIK cell, a new immunologically competent $CD3^+CD56^+$ cell, exhibited high cytotoxicity against tumor cells. In addition, it displayed characteristics of T lymphocytes and NK cells, which can kill cancer cells efficiently. ii) DC cells, the most effective antigen-presenting cells, could induce the immune activity of antitumor cells under the effect of tumor antigen presentation, which activated T cell responses. Meanwhile, the existence of DC cells could enhance the tumor-killing efficiency of CIK cells. Our proposal was supported by the results, among which the numbers of CD3-, CD4-, CD8- and NK-expressing cells in the study group were remarkably higher than the baseline levels. Meanwhile, improved life quality was reported in the study group. These findings demonstrated that higher functional activity was noted in patients after transfusion DC cell-CIK cell transfusion. Regarding clinical efficacy, a higher CR rate was noted in the study group than in the control group. However, no statistical difference was identified in our study. Further large-scale studies are needed to investigate the clinical efficacy of IMRT plus DC-CIK immunotherapy.

To the best of our knowledge, DC-CIK therapy has been used for treating patients with NSCLC, as it could activate CIK cells to enhance antitumor effects in these patients (Gerard and Debruyne, 2009; Yuan et al., 2013). For example, in a study that investigated the efficacy of DC-CIK immunotherapy in treating metastatic NSCLC, the combination of cryotherapy, chemotherapy, and DC-CIK therapy proved most effective for treating metastatic NSCLC (Yuan et al., 2013). A literature review indicated that few studies have investigated the

treatment efficacy of DC-CIK therapy in patients with EC. We speculated that the incidence of EC is comparatively low worldwide. Therefore, it is difficult to collect a large sample size to investigate the treatment efficacy. Interestingly, a high incidence of EC has been reported in Henan Province, which is located in northern China (Ma et al., 2009). For the elderly population, immunotherapy has been commonly preferred because of its advantages including promoting immune response and inducing low toxicity. In our study, few side effects were reported in elderly patients with EC. In the study group, 3 patients reported fever, whereas 12 patients exhibited excitation after DC cell-CIK cell transfusion. Among the 12 excited patients, 4 patients reported insomnia, which was speculated to be related with IL-2, cellular health, psychological factors, and the radiotherapy dose. Compared with the findings in the control group, a lower incidence of bone marrow suppression was noted in the study group. Meanwhile, no severe organ injuries were reported in the study group.

In conclusion, DC-CIK therapy plus radiotherapy was safe and effective in treating EC in elderly patients. Further large trials are needed to investigate its potential mechanism.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENT

We thank the anonymous reviewers for reviewing this manuscript.

REFERENCES

Cui Y, Yang X, Zhu W, Li J, et al. (2013). Immune response, clinical outcome and safety of dendritic cell vaccine in combination with cytokine-induced killer cell therapy in cancer patients. *Oncol. Lett.* 6: 537-541.

Faiz Z, Lemmens VE, Siersema PD, Nieuwenhuijzen GA, et al. (2012). Increased resection rates and survival among patients aged 75 years and older with esophageal cancer: a Dutch nationwide population-based study. *World J. Surg.* 36: 2872-2878.

Gerard C and Debruyne C (2009). Immunotherapy in the landscape of new targeted treatments for non-small cell lung cancer. *Mol. Oncol.* 3: 409-424.

Li H, Wang C, Yu J, Cao S, et al. (2009). Dendritic cell-activated cytokine-induced killer cells enhance the anti-tumor effect of chemotherapy on non-small cell lung cancer in patients after surgery. *Cytotherapy* 11: 1076-1083.

Ma YT, Lian SY, Liu ZC, Cheng LP, et al. (2009). Period survival analysis of esophageal cancer in Linzhou city of Henan province. *Zhonghua Yu Fang Yi Xue Za Zhi* 43: 1100-1104.

Shi SB, Ma TH, Li CH and Tang XY (2012). Effect of maintenance therapy with dendritic cells: cytokine-induced killer cells in patients with advanced non-small cell lung cancer. *Tumori* 98: 314-319.

Son SH, Song JH, Choi BO, Kang YN, et al. (2012). The technical feasibility of an image-guided intensity-modulated radiotherapy (IG-IMRT) to perform a hypofractionated schedule in terms of toxicity and local control for patients with locally advanced or recurrent pancreatic cancer. *Radiat. Oncol.* 7: 203.

Wang LD, Zhou Q, Feng CW, Liu B, et al. (2002). Intervention and follow-up on human esophageal precancerous lesions in Henan, northern China, a high-incidence area for esophageal cancer. *Gan To Kagaku Ryoho* 29 (Suppl 1): 159-172.

Yang L, Ren B, Li H, Yu J, et al. (2013). Enhanced antitumor effects of DC-activated CIKs to chemotherapy treatment in a single cohort of advanced non-small-cell lung cancer patients. *Cancer Immunol. Immunother.* 62: 65-73.

Yuan Y, Niu L, Feng M, Wang X, et al. (2013). Therapeutic outcomes of combining cryotherapy, chemotherapy and DC-CIK immunotherapy in the treatment of metastatic non-small cell lung cancer. *Cryobiology* 67: 235-240.