Original Paper

Phase II/III Study of Radiofrequency Ablation Combined with Cytokine-Induced Killer Cells Treating Colorectal Liver Metastases

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Key Words

Colorectal liver metastases • Radiofrequency ablation • Cytokine-induced killer cells • Progression-free survival • Overall survival

Abstract

Purpose: This phase II/III, non-randomized clinical trial aimed to determine the efficacy and safety of the combination of radiofrequency ablation (RFA) and cytokine-induced killer (CIK) cells transfusion for patients with colorectal liver metastases (CRLMs). **Experimental Design:** A total of 60 eligible patients with CRLMs were enrolled and divided into Group A (RFA alone, n = 30) and Group B (RFA plus CIK, n = 30), and following enzyme-linked immunosorbent spot assay was performed in 8 patients with CEA > 50 ng/mL pre-RFA and 7 days post-RFA and CIK treatment, respectively. **Results:** The median progression-free survival (PFS) times of Group A and Group B were 18.5 months and 23 months, respectively ($P = 0.0336$). The 3-year progression-free rates were 13.3% in Group A and 20.3% in Group B, respectively. The median overall survival time was 43 months in Group A, and not reached in Group B. The 3-year survival rates were 64.6% in Group A and 81.0% in Group B, respectively ($P = 0.1187$). Among the 8 patients with CEA > 50ng/mL, 6 had increase of circulating CEA-specific T cells after RFA ($P = 0.010$). After CIK cell therapy, the number of CEA-specific T cells increased in all the 8 patients comparing with that pre-treatment ($P = 0.001$) and in 7 patients comparing with that post-RFA ($P = 0.028$). **Conclusions:** We firstly confirm that the combination of RFA and CIK cells boosts CEA-specific T cell response and shows to be an efficacious and safe treatment modality for patients with CRLMs.

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Introduction

Colorectal cancer occupies the third place of solid malignancies around the world with over 694,000 deaths (8.5% of all cancer-related deaths) in 2012 [1]. In China, both the incidence rate and mobility rate have been increasing in the recent decades [2]. It is estimated that 50% - 60% of colorectal cancer patients develop liver metastases eventually [3, 4]. These colorectal liver metastases (CRLMs) are the major reason resulting in failure to treatment [5]. Though hepatic resection is considered as the standard therapy for curing CRLMs, 75% - 85% of patients with liver-only metastases are evaluated as unresectable [6, 7], especially in the absence of symptoms [8]. Thus, novel approaches for curing CRLMs are urgently necessary [9, 10].

In recent years, immunotherapy has emerged as an efficacious treatment modality with encouraging efficacy and slight adverse events (AEs) in cancer therapy [11]. CIK cells are a subset of T lymphocytes with a natural killer T cell phenotype expressing both CD3 and CD56, and present potent non-major histocompatibility complex-restricted cytotoxicity against multiple tumors [12-17]. CIK cell therapy has been evaluated for cancer patients in a number of clinical trials [18-27].

Besides, previous studies have suggested that radiofrequency ablation (RFA) has promising efficacy treating CRLMs [28-32]. However notably, the high temperature of RFA can lead to coagulation necrosis of CRLM tissue and furthermore recover the immune suppression by reducing the tumor burden [32, 33]. And infused immune cells can restore the impaired immunity and thus produce a synergistic action. Therefore, we propose that the combination of CIK cell therapy and RFA can produce synergic antitumor effect for patients with CRLMs.

There have not been any similar articles, so we designed this study to investigate the efficacy and safety of the combination of RFA and CIK cell transfusion for patients with CRLMs.

Patients and Methods

Study design

This is a phase II/III, parallel assignment, open label, non-randomized clinical trial registered in <https://www.clinicaltrials.gov> (ClinicalTrials.gov identifier: NCT 02419677) and approved by the Ethics Committee of The Third Affiliated Hospital of Soochow University (also named The First People's Hospital of Changzhou). This study complied with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines. All patients provided written informed consents before the enrollment.

The inclusion criteria included 1) age ranging from 18 to 75 years old; 2) diagnosis of histologically proved colorectal adenocarcinoma; 3) had having complete resection of primary lesions in color or rectum; 4) existence of liver metastases resectable or accessible for radiofrequency ablation (5 or less lung metastatic lesions with the size no larger than 5 cm); 5) serum bilirubin level no larger than 2.0 mg/dl; 6) ECOG performance status (PS) of 0 or 1; and 7) expected survival of 1 year or longer. The exclusion criteria included patients 1) with extrahepatic metastatic lesions; and 2) with other neoplastic diseases other than colorectal cancer.

According to receiving CIK cell therapy or not, the patients were divided into Group A (RFA alone) and Group B (RFA plus CIK therapy).

The primary endpoint was progression-free survival (PFS) time defined as the time from the date of RFA to the date of disease progression inside or outside the liver, or the date of the latest follow-up. The secondary endpoint was overall survival (OS) time defined as the time from the date of RFA to the date of death from any cause, or the date of the last follow-up.

RFA

The indication of RFA was discussed by the Colorectal Cancer Collaborative Group (CCCG) consisting of clinical oncologists, interventional specialists, radiologists, immunologists and surgeons in our institute.

The multidisciplinary assessment included the potential benefits of RFA in removing tumor mass and improving quality of life and also the reduction of the complications of RFA.

For all the patients in both Group A and Group B, RFA was performed percutaneously under computed tomography (CT)/ultrasound guidance. All the operations were performed using the RITA 1500 generator (RITA Medical Systems Inc., Mountain View, CA, USA) composed of a generator that provided 150 W of power and a multitined expandable electrode (StarBurst XL, RITA) consisting of a 15-gauge insulated cannula and 9 individual electrodes tines of 10 to 15 cm in length. It was deployed *in situ* after ultrasound-guided placement of the needle electrode into the target tumor. If tumors were less than 3.0 cm in diameter, the electrode was deployed into the center of the tumor. Each operation of RFA energy maintained 15-25 minutes to form an ablation zone of about 5.0 cm in diameter. If tumors were larger than 3.0 cm, multiple overlapping ablation zones were necessary for tumor destruction and a surrounding rim of non-tumor liver tissue. If patients had more than 1 lesion, each tumor was ablated separately.

CIK cell therapy

All patients in Group B received CIK cell therapy. Seven days before RFA, peripheral blood mononuclear cells (PBMCs) were collected. Separation of PBMCs, induction of CIK cells and cytotoxic activity detection were done as described previously [34]. Seven days after RFA more than 1×10^9 autologous CIK cells were transfused into the patient via superficial vein within 1 hour. Five transfusions were defined as 1 cycle. The first transfusion in the first cycle was given on day 7 following RFA and each of the 5 transfusions in 1 cycle were performed once every 2 days, which means the 5 transfusions were administered on day 7, 9, 11, 13 and 15, respectively. All treatment-related AEs were observed and recorded. See Figure 1.

Enzyme-linked immunosorbent spot (ELISPOT) assay

Detection of TAA-specific T- cell response was performed as described [35]. Briefly, PBMCs were stimulated with CEA peptides [CEA: 691-699 (IMIGVLGV), CEA: 605-613 (YLSGANLNL), and CEA: 694-702 (GVLGVALL)] (7 μ g/mL each), and positive control peptides [(anti-CD3 mAb CD3-2 (Mabtech) 100 ng/mL]. Positive control consisted of 1 mmol/L of phorbol 12-myristate 13-acetate and 10 ng/mL of Ionomycin. No antigen was added to the negative control well. K562/*A 0201 cells were used as antigen-presenting cells. ELISPOT plates (ELISpot plus kit, Sweden) were washed 4 times with sterile PBS (200 μ L/well) and incubated with medium containing 10% serum for 30 minutes at room temperature. Peptide-pulsed antigen-presenting cells were incubated for 2 to 3 h at 37°C in a 5% CO₂ incubator, washed, and resuspended at 5×10^5 /mL, and 100 μ L were added to the ELISPOT wells for a final cell concentration of 5×10^4 per well. After overnight incubation, PBMCs were counted and the concentration was adjusted to 1×10^6 /mL, and 100 μ L were transferred to appropriate wells on the ELISPOT plate. The number of individual spots per well was counted using an ELISPOT Plate Reader (iSPOT, German). Mean IFN- γ responses to each of the antigens and controls were calculated after subtraction of background IFN- γ responses obtained from the negative control wells. ELISPOT assay were performed before treatment, 7 days after RFA and 7 days after CIK cell transfusion, respectively.

Follow-up

Follow-up was performed every 3 months after RFA as the same in 2 groups for a median period of 26 months. Follow-up consisted of physical examination, CT scan, tumor markers and other laboratory examinations. Disease recurrence was diagnosed based on physical and radiological examinations, and routine histological examinations were carried out as needed. Besides, AEs were also recorded.

Fig. 1. Treatment schedule. Seven days before RFA (-Day 7), peripheral blood mononuclear cells are collected and separated. Seven days after RFA (Day 7), autologous CIK cells are transfused into the patient. Five transfusions were defined as 1 cycle. The first transfusion in the first cycle was given on day 7 following RFA and each of the 5 transfusions in 1 cycle were performed once every 2 days (Day 7, 9, 11, 13 and 15). Later patients are followed up.

Statistical analysis
Categorical and numerical variables were compared by chi-square and Student *t* tests. The PFS and OS curves were estimated using the Kaplan–Meier method. Paired-sample *t* test was used for comparing IFN- γ spot tested by ELISPOT pre- and post-treatment. A *P* value less than 0.05 was considered statistically significant. Data were analyzed using SPSS software (v16.0, SPSS Inc.).

Table 1. Patient baseline characteristics (n = 60). Group A: radiofrequency ablation alone; Group B: radiofrequency ablation plus cytokine-induced killer cells

Characteristics	Total (n = 60)	Group A (n = 30)	Group B (n = 30)	<i>P</i>
Age (year)				0.662
Median	54	55	54	
Range	38-75	38-74	43-75	
Sex				0.789
Male	38	18	20	
Female	22	12	10	
Performance status				0.333
0/1	48	26	22	
2	12	4	8	
Time to liver metastases				1.000
Synchronous	19	9	10	
Metachronous	41	21	20	
Site of primary cancer				0.422
Colon	38	21	17	
Rectum	22	9	13	
Number of lesions				0.781
1	35	17	18	
2	15	7	8	
3	10	6	4	
Size of largest lymphnode				0.792
≤ 3 cm	24	13	11	
> 3 cm	36	17	19	
Baseline CEA value				0.589
< 200 ng/ml	39	18	21	
> 200 ng/ml	21	12	9	
History of chemotherapy				0.761
Yes	45	22	24	
No	15	8	6	

Results

Patient enrollment

Between January 2010 and December 2014, a total of 60 eligible patients with CRLMs were enrolled and divided into Group A (n = 30) and Group B (n = 30). The details of the patients' clinicopathological characteristics are shown in Table 1. There were no statistically significant differences between the characteristics including age, sex, PS score, time to liver metastases, site of primary cancer, number of lesions, size of the largest node, baseline value of CEA, prior chemotherapy history in the 2 groups.

Immunological effect of combination of RFA and CIK cell therapy

In order to observe the immunological effect of antitumor treatment on T-cell response, detection of CEA-specific T cells was performed by direct *ex vivo* analysis (IFN- γ ELISPOT assay) in 8 patients with CEA > 50 ng/mL. Positive T cell responses against each CEA-derived peptide were observed in 6 patients after RFA ($P = 0.010$). After CIK cell therapy, the number of CEA-specific T cells increased in all the 8 patients comparing with that pre-treatment ($P = 0.001$) and in 7 patients comparing with that post-RFA ($P = 0.028$). See Figure 2.

PFS and OS

The median PFS times of Group A and Group B were 18.5 months and 23 months, respectively ($P = 0.0336$). The 3-year progression-free rates were 13.3% in Group A and 20.3% in Group B, respectively. By the end of follow-up, 9 patients in Group A and 6 patients

Fig. 2. ELISPOT assay pre/post-treatment. Eight patients with CEA > 50 ng/mL were selected. ELISPOT assay are performed before treatment, 7 days after RFA and 7 days after CIK cell transfusion, respectively. Among the 8 patients with CEA > 50 ng/mL, 6 have increase of IFN- γ spot more than 5 spots 7 days after RFA (but before CIK cell infusion, $P = 0.010$), 7 have increase 7 days after CIK cell infusion ($P = 0.028$). All patients have increase of IFN- γ spot 7 days after CIK cell transfusion as comparing to pre-treatment ($P = 0.001$).

Fig. 3. A. The median PFS times of Group A and Group B are 18.5 months and 23 months, respectively. There is a statistically significant difference between the PFS times ($P = 0.0336$). B. The median OS time is 43 months in Group A, and not reached in Group B. There is not a statistically significant difference ($P = 0.1187$).

Fig. 4. Contrast enhanced CT images of a female patient with solitary liver metastasis, who has no recurrence 3 years after radiofrequency ablation and CIK cell transfusion.

in Group B had died. The median OS time was 43 months in Group A, and not reached in Group B. The 3-year OS rates were 64.6% in Group A and 81.0% in Group B, respectively ($P = 0.1187$). See Figure 3. The images of a typical case are shown in Figure 4.

Treatment-related AEs

Generally, there were no severe AEs during the treatment. Only 2 patients in Group B had transient low fever and both recovered after symptomatic management. There was no AE in Group A.

Subsequent treatment

Eight patients in Group A and 10 in Group B received RFA again due to *in situ* recurrence of CRLMs.

Discussion

There has been no report focusing on the combination of RFA and CIK cells infusion treating CRLMs so far. The primary objective was to evaluate whether CIK cells can boost the efficacy of RFA for patients with resectable CRLMs and expected results were observed. RFA obliterated tumors completely in all patients so assessment of tumor response was not applicable. No matter survival times or AEs, the results suggest that the combination of RFA and CIK cell therapy is a better option than RFA alone.

The RFA-induced antitumor immune responses have been shown in animal models and clinical settings [36-38], and confirmed again by our ELISPOT assay results. However, the

immune reactions elicited by RFA alone are stimulated at a low level and last only a short time [39]. So the immune reactions requires amplification to control tumor progression subsequent to ablation by immune cells [38] including CIK cells and others such as dendritic cell-based hyperthermic tumor vaccine [40] and CEA- and gp70-specific T cells [36]. Relatively, CIK cell therapy has been studied more often and the transfusion of CIK cells has widely been used clinically.

Generally, RFA plays a role as minimally invasive surgical approach similar to surgical resection of CRLMs. Besides, RFA increases the exposure of tumor cell epitopes and promotes the synthesis of heat shock protein to effectively activate antitumor immune responses [41]. The cytokine and/or chemokine milieu can also enhance the antineoplastic function and promote the recruitment of circulating immune cells to tumor site [42]. Our ELISPOT assay results show that CIK cell therapy can boost the CEA-specific response after RFA. In this sense, the modality of RFA plus CIK cell therapy is also a combination of 2 kinds of immunotherapies with different mechanisms. Previously in an animal model, we found that CIK cells co-cultured with dendritic cells loaded with the protein lysate produced by RFA induced a specific antitumor response to colon carcinoma [12]. Recently, we found that RFA of liver metastases increased T cell infiltration in primary colorectal tumor [43]. Therefore, we conclude that CIK cell therapy and RFA can produce a synergistic antitumor effect.

The difference between the PFS times in the 2 groups was statistically significant but only a borderline *P* value was obtained. One reason might be due to the relatively small sample size and short time span of follow-up. And CIK cell therapy is only an adjuvant treatment modality for CRLMs. Furthermore in practice, patients sometimes have to be scheduled for receiving the infusion, resulting in postponing of treatment plan. While some series have reported that RFA superior to surgical resection, others have also shown RFA to be equivalent or even inferior in OS [37, 44-47]. Therefore, it is reasonable to propose that RFA plus CIK cell therapy is superior to surgical resection and produces lesser AEs. Anyway, the prolonged PFS time was attributed to the adjuvant CIK cell therapy, which is the most important finding of our study.

Several studies have shown that RFA induced tumor antigen-specific T cell response detected by ELISPOT assay in peripheral blood [48, 49]. Mizukoshi et al. showed that the number of TAA-specific T cells was associated with the PFS in HCC patients [49]. Here, we obtained the complete data of ELISPOT including pre-RFA, post-RFA, and post-CIK only in 8 patients. These limited data were not enough to justify the association between the strength of immune response and survival.

Conclusively, we have confirmed that CIK cell therapy can boost the tumor antigen-specific response induced after RFA and the combination of RFA and CIK cell therapy is an efficacious and safe treatment modality for patients with CRLMs. The progressive clinical trials with larger samples and longer follow-up period are warranted.

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Disclosure Statement

The authors declare that they have no conflict of interest.

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