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ORIGINAL ARTICLE

Effect of preoperative infusion chemotherapy combined with hyperthermia on sPD-L1 and CEA levels and overall survival of elderly patients undergoing radical resection of lung cancer

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Summary

Purpose: *To explore the effect of preoperative infusion chemotherapy combined with hyperthermia on the expressions of sPD-L1 and CEA in elderly patients undergoing radical surgery for lung cancer, and their prognosis.*

Methods: *136 elderly patients undergoing radical resection of lung cancer in our hospital from October 2012 to October 2014 were studied. Patients were randomly divided into two groups, namely the combination group and the individual treatment group, with 68 patients in each group. Patients in the individual treatment group received only preoperative chemotherapy, whereas those in combination group received preoperative infusion chemotherapy and preoperative and postoperative hyperthermia. The treatment efficacy, levels of sPD-L1 tumor marker CEA (carcino-embryonic antigen), and T-lymphocyte subsets ($CD4^+$, $CD3^+$, $CD8^+$, $CD29^+$) were compared between the two groups. Three-year follow-up data were collected to compare the overall survival (OS) of the two groups.*

Results: *The effective rates in the combination group and individual treatment group were 87.5 and 67.5%, respectively ($p<0.05$). After treatment, lower serum levels of CEA and sPD-L1 were seen in the combination group vs the individual treatment group ($p=0.036$, $p=0.008$, respectively). Levels of T-lymphocyte subsets $CD4^+$, $CD3^+$, and $CD29^+$ in both groups increased, and were higher in the combination group vs the individual treatment group ($p<0.05$). Follow-up data demonstrated that OS in the combination group and the individual treatment group was 61.7 and 48.5%, respectively. Significant difference in OS between the two groups was confirmed by Log-rank test ($p=0.043$).*

Conclusions: *Preoperative infusion chemotherapy combined with hyperthermia for elderly patients with lung cancer can improve patient immunity, inhibit tumor growth and lengthen overall survival by improving T-lymphocyte subset levels and reducing the circulating tumor cell content.*

Key words: *hyperthermia, infusion chemotherapy, lung cancer, overall survival therapeutic effect*

Introduction

Lung cancer is a malignant tumor with high incidence. Due to the increased air pollution today, the global incidence of lung cancer has been risen year by year. It is reported that the elderly are susceptible to lung cancer, and account for 30 to 40% of the total lung cancer patients [1]. Since most elderly patients also suffer of basic diseases, such as diabetes, hypertension and heart disease, radiotherapy and chemotherapy are preferred options for elderly lung cancer patients because of poor surgical tolerance [2].

At present, inhibition of proliferation of cancer cells and induction of apoptosis have become the main adjuvant treatment strategies for lung cancer

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[3]. Preoperative hyperthermia combined with infusion chemotherapy exerts an ideal adjuvant effect by directly inducing apoptosis of tumor cells. However, the therapeutic mechanism of this technique is rarely reported. Programmed death-1 ligand-1 (PD-L1), a member of the negative co-stimulatory molecule family, is involved in tumor immune escape. PD-L1 is present in many human tumor cell lines and solid tumor tissues [4]. CEA is closely correlated to chemotherapy efficacy and long-term survival in advanced lung cancer [5].

Therefore, we detected sPD-L1 and CEA levels before and after the treatment to examine the therapeutic effect of preoperative hyperthermia combined with infusion chemotherapy. Our study provides a clinical basis for further exploring the therapeutic mechanism of preoperative hyperthermia combined with infusion chemotherapy for elderly patients undergoing radical resection of lung cancer.

Methods

Subjects

We studied 136 elderly patients undergoing radical resection of lung cancer in our hospital from October 2012 to October 2014, including 63 males and 73 females, with an average age of 73.5 ± 4.4 years. Inclusion criteria were the following:(1) Lung cancer confirmed by cytological or pathological examination; (2) Over 65 years old; (3) No contraindication to surgery; (4) No chemotherapy contraindications; (5) Lung cancer with TNM stage II-III [6]. Exclusion criteria included: (1) Cardiopulmonary dysfunction; (2) Patients with malignant tumors within 5 years; (3) Incomplete clinical data; (4) Predicted OS less than 1 month; (5) Less than 6 months of follow-up duration.

This study was approved by the Ethics Committee of the Affiliated Huai'an No.1 People's Hospital of Nanjing Medical University. All patients signed informed consent.

Therapeutic methods

Patients in the individual treatment group underwent hyperthermic preoperative infusion chemotherapy. One week before the radical lung resection, angiography of the intercostal or subclavian and bronchial artery was performed. The intubation position of the supply artery of tumor was determined by the angiographic results. Doses of chemotherapeutic drugs (mitomycin and cisplatin) depended on body surface area and physical condition. Patients in the combination group received hyperthermic preoperative infusion chemotherapy. High-frequency hyperthermia was performed 1 hr prior to chemotherapy (Hejia Medical Equipment Co., Ltd., Zhuhai city, model HG-2000, Zhuhai, China). Hyperthermia was performed with 13.56 Hz frequency, temperature 41-44°C, voltage 200 V, and treatment duration 1 hr. Twenty four hrs after the infusion chemotherapy, hyperthermia was performed again in the same way. All patients received chemotherapy once every 3 weeks for a total of 4 cycles. Survival curves were drawn based on 3-year follow-up data.

Evaluation

After 3 months of treatment, the therapeutic effect on lung cancer patients was evaluated. Complete remission (CR) was considered as complete tumor disappearance with CEA returning to normal level; partial remission (PR) was considered as the reduction of maximum vertical diameters and product of maximum diameter of tumor to over 50%; stable disease (SD) was considered as the reduction of maximum vertical diameter and product maximum diameters to 25-50%, and/or tumor increase less than 25% of the product of the original diameter; progressive disease (PD) was considered increase of the maximum vertical diameter and product maximum diameter over 25% and also the appearance of new lesion (s). Effectiveness was defined as the sum of CR+PR rate [7]. CEA levels were measured in venous blood samples before and after 3 months of treatment. The content of sPD-L1 in peripheral blood was measured by enzyme-linked immunosorbent assay (ELISA) kit. $CD8^+$, $CD4^+$, $CD3^+$, and $CD29^+$ in serum were detected by immuno-

Table 1. Basic characteristics of enrolled subjects

Characteristics	*Combination group*	*Individual group*	*p*
Age(years)	72.7 ± 3.1	74.8 ± 4.4	0.442
Gender			0.605
Male	33	30	
Female	35	38	
BMI	17.6±3.6	18.2±2.8	0.374
Pathologic types			0.603
Squamous cell carcinoma	31	28	
Adenocarcinoma	37	40	
TNM			0.662
II	56	54	
III	12	14	

fluorescence. OS was recorded and assessed according to the follow-up data.

Statistics

Statistical analyses were performed using the SPSS 19.0 software (IBM, Armonk, NY, USA). Quantitative data were expressed as mean ± standard deviation and analyzed by t-test. Qualitative data were expressed as percents and analyzed by x^2 test. Kaplan-Meier method and Log-rank test were used for survival analysis. Statistically significant difference was set at *p<0.05 and **p<0.01.

Results

Basic patient characteristics

This study included 136 patients older than 65 years undergoing radical resection of lung cancer in our hospital from October 2012 to October 2014. The patients were randomly assigned into the combination group and the individual treatment group, with 68 patients in each group. In the combination group there were 33 males and 35 females with average age of 72.7 ± 3.1 years (range 66-86). Fifty six patients had stage IIIB and 12 stage IV. In the individual treatment group, there were 30 males and 38 females with average age of 74.8 ± 4.4 years (range 65-87). Fifty four patients had stage IIIB and 14 stage IV. No significant differences in sex, age, BMI, TNM staging and pathological types were found between the two groups (p>0.05, Table 1).

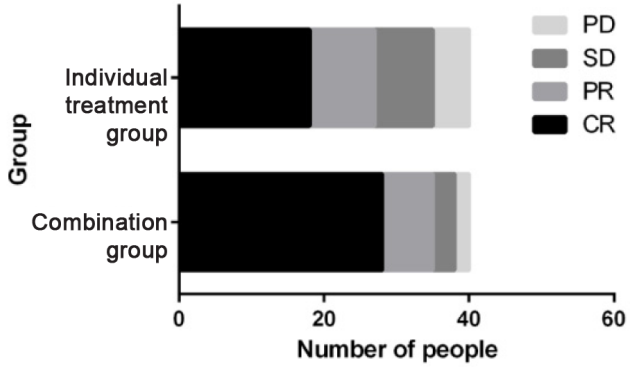

Figure 1. Comparison of therapeutic effect between the two groups. The clinical efficacy in the combination group was significantly higher compaared with the individual treatment group (p=0.032).

Comparison of therapeutic effect

The total effective rate in the combination group and the individual treatment group was 87.5% and 67.5%, respectively. The clinical efficacy was significantly different between the two groups (p=0.032, Figure 1).

Comparison of tumor-related indicators

Serum CEA levels were significantly lower in both groups post-treatment. CEA level in the combination group was significantly lower than in those of individual treatment group (p=0.036). Pretherapeutic sPD-L1 level was not significantly different between the two groups. After different treatment methods, sPD-L1 levels in both groups decreased, which was more obvious in the combination group (p=0.008, Figure 2).

Changes in T-lymphocyte subsets

No remarkable differences in pretherapeutic levels of $CD4^+$, $CD3^+$, $CD8^+$ and $CD29^+$were found between the two groups. After 3 months of treatment, $CD8^+$level decreased, whereas the levels of $CD4^+$, $CD3^+$ and $CD29^+$ increased in both groups (p<0.05). Lower $CD8^+$ level and higher $CD4^+$, $CD3^+$ and $CD29^+$ level were found in the combination group than in those of individual treatment group (p<0.05, Figure 3).

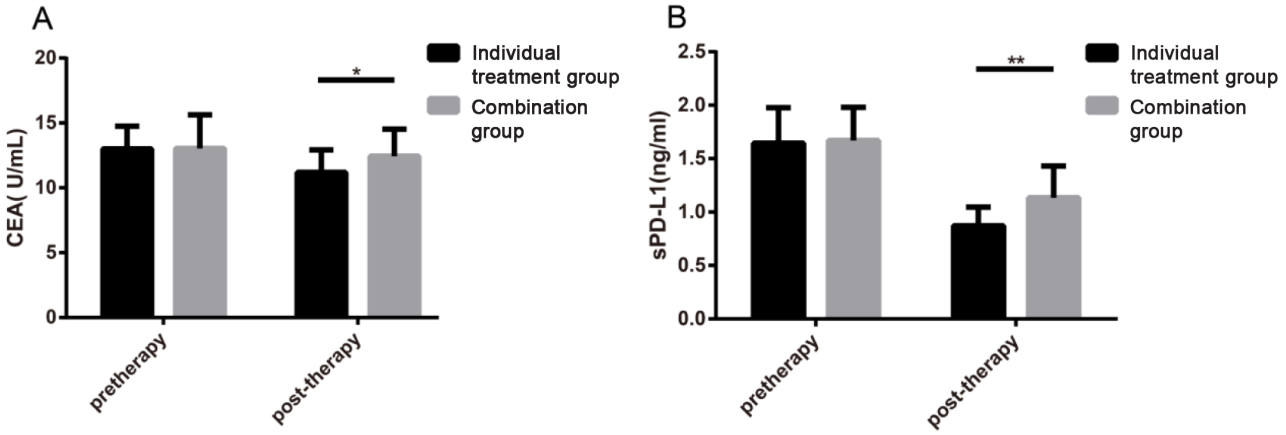

Figure 2. Comparison of tumor-related indicators between the two groups of patients. **(A)** There was no significant difference in CEA level between the two groups before treatment. **(B)** After treatment, the sPD-L1 level of the combination group was lower than that of the individual treatment group. *p<0.05, **p<0.01.

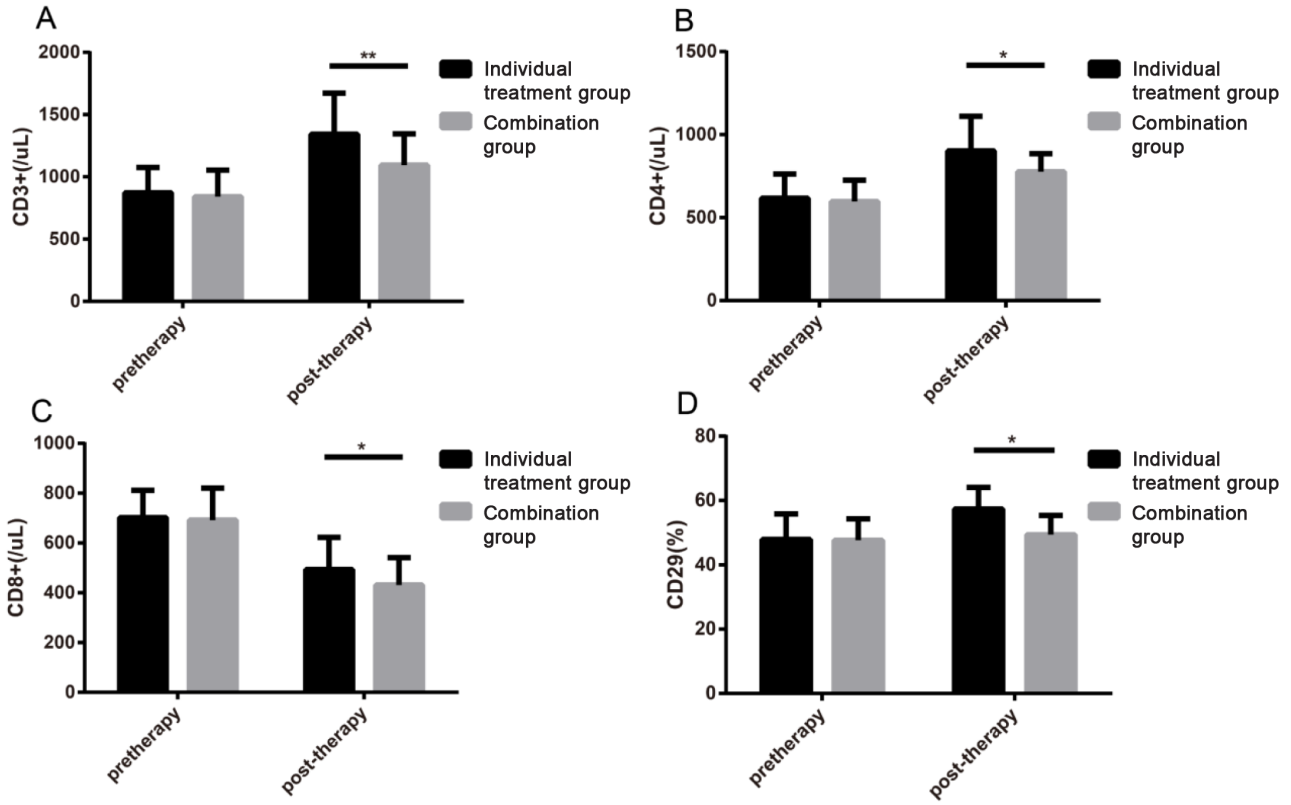

Figure 3. Changes of T-lymphocyte subsets before and after the treatment in both groups. **(A)** After treatment, there were more $CD3^+$ lymphocytes in the combination group than in the individual treatment group. **(B)** After treatment, there were more $CD4^+$ lymphocytes in the combination group than that of individual treatment group. **(C)** After treatment, there were more $CD8^+$ lymphocytes in the combination group than in the individual treatment group. **(D)** After treatment, there were more $CD29^+$ lymphocytes in the combination group than in the individual treatment group. *p<0.05, **p<0.01.

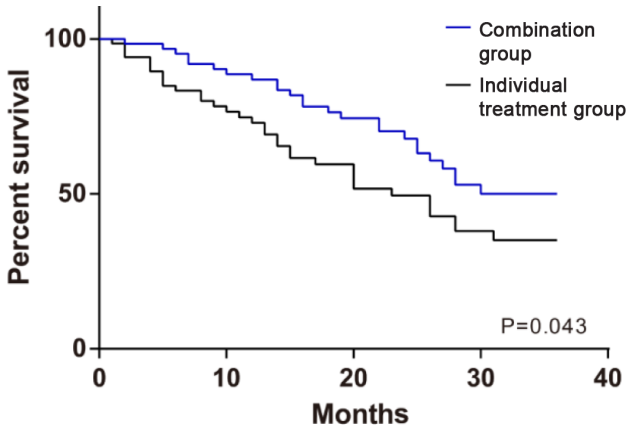

Figure 4. After 3 years of follow-up, the survival time of the combination group was significantly longer compared with individual treatment group.

Comparison of overall survival

Three-year follow-up patient data were collected for survival analyses. The OS rate was 61.7% in the combination group and 48.5% in the individual treatment group. Log-rank test suggested that OS between the two groups was statistically different (p=0.043, Figure 4).

Discussion

Hyperthermia has become an effective method for tumor treatment in recent years [8,9]. It mainly increases the local temperature of tumor tissue through heating and promotes tumor cell apoptosis. Preoperative infusion chemotherapy combined with hyperthermia can effectively improve the therapeutic effect of cancer patients. Chemotherapeutic drugs can reduce tumor tissue volume and inhibit tumor cell metastasis. Therefore, combination of the two methods can synergistically improve the therapeutic effect of cancer patients [10]. This study showed that the effective rate in the combination group was 87.5%, which was remarkably higher than that of the individual treatment group and this study confirmed that preoperative infusion chemotherapy combined with hyperthermia can effectively improve the treatment results of elderly patients with lung cancer.

The occurrence, development and prognosis of lung cancer are closely related to tumor immune microenvironment and immune function of the body [11,12]. sPD-L1 is mainly expressed on the surface of natural killer cells, activated B-lympho-

cytes and T-lymphocytes. It negatively regulates immune response by inhibiting T and B cell functions via PD-1/sPD-L1 pathway [13,14]. Relevant reports [15] showed that t sPD-L1 level was higher in lung cancer patients than in healthy individuals. sPD-L1 level was correlated to distant metastasis and regional lymph node metastasis of lung cancer cells, suggesting that sPD-L1 can serve as a predictive indicator of lung cancer. Our results revealed lower sPD-L1 level in the combination group than in the individual treatment group after treatment. The reason may be explained by the sensitizing effect of infusion chemotherapy and induction of apoptosis of tumor cells through heating [16]. In addition, hyperthermia can also increase the sensitivity of tumor cells to chemotherapeutic drugs within a certain range. Hyperthermia activates HIF-1α expression in lung cancer through AKT and ERK pathways, thereby enhancing the sensitivity of tumor cells to chemotherapeutic drugs [17-19]. Hyperthermia also improves the body's immunity by downregulating sPD-L1 and suppressing the negative effects on the immune response.

In addition, this study also found lower $CD8^+$ levels and higher $CD4^+$, $CD3^+$ and $CD29^+$ levels after treatment in the combination group, further suggesting that infusion chemotherapy combined with hyperthermia not only modulates tumor antigens, but also regulates the body's immune function [20]. We consider that tumor tissues in the combination group were controlled by this therapeutic method. T-lymphocyte subsets exert key roles in specific cell immune killing and regulation of the body's immune function [21]. Therefore, the investigation of peripheral blood lymphocyte subsets is of great significance to guide immunotherapy, monitor tumor recurrence and evaluate prognosis. In addition to the classical T-lymphocyte subsets, other T-lymphocytes also participate in cellular immunity. CD29 is a receptor for a variety of extracellular matrix proteins that induces T cells [22]. Inflammatory reaction activates $CD4^+$ and $CD29^+$ cells, which in turn induce Th1 cytokines to secrete tumor necrosis factor α and γ interferon. Therefore, we confirmed that lung cancer patients undergoing combination therapy present higher immunity.

Tumor markers are frequently used in the evaluation of radiotherapy and chemotherapy effects. CEA is closely related to the occurrence, development, metastasis, and infiltration of lung cancer [23,24]. In the present study CEA level in both groups was remarkably decreased, which was more obvious in the combination group. According to the 3-year follow-up data, OS in the combination group was longer than that of the individual treatment group, suggesting that preoperative infusion chemotherapy combined with hyperthermia improves lung cancer prognosis.

However, this study had some limitations. Firstly, it was a single-center retrospective analysis with a limited sample size. Secondly, the follow-up duration was short. Further investigations with multi-center prospective randomized studies with a large sample size are surely required.

Conclusions

Preoperative infusion chemotherapy combined with hyperthermia can reduce sPD-L1 level and improve the immune response, reducing the CEA level and prolonging the survival of patients undergoing radical resection of lung cancer.

Conflict of interests

The authors declare no conflict of interests.

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