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Original Article

Effects of ginsenoside Rg3 combined with ^{125}I seeds on serum TGF-α, TGF-β1, and VEGF in patients with medium and advanced non-small cell lung cancer

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Abstract: Objective: The aim of this study was to investigate the effects of ginsenoside Rg3 combined with ^{125}I seeds on serum transforming growth factor-α (TGF-α), TGF-β1, and vascular endothelial growth factor (VEGF) in patients with medium and advanced non-small cell lung cancer (NSCLC). Methods: A total of 62 patients with medium and advanced NSCLC, treated from June 2015 to May 2017, were selected and randomly divided into a control group (n = 31) and observation group (n = 31). Patients in the control group were treated with chemotherapy alone (gemcitabine + platinol (GP) regimen), while those in observation group, based on the GP regimen, were treated with ginsenoside Rg3 combined with ^{125}I seed implantation. Serum levels of serum TGF-α, TGF-β1, and VEGF in patients, before and after treatment, were detected via ELISA. Results: At 4, 8, and 16 weeks after treatment, serum VEGF levels in patients in the two groups were significantly decreased, compared with those before treatment, with more decreases noted in the observation group ($p < 0.05$). At 4, 8, and 16 weeks after treatment, serum levels of TGF-α and TGF-β1 in the control group were not changed significantly ($p > 0.05$), but they were significantly decreased in the observation group, compared with those before treatment ($p < 0.05$). Conclusion: In conclusion, ginsenoside Rg3 combined with ^{125}I seed implantation can improve patient immunity, reduce toxicity, and enhance efficacy of routine chemotherapy, as well as inhibit TGF-α, TGF-β1, and VEGF expression.

Keywords: Ginsenoside Rg3, ^{125}I seed implantation, NSCLC, immune function, TGF-α, TGF-β1, VEGF

Introduction

Lung cancer is one of the most common malignant tumors found in clinical practice. Its incidence and mortality rates are ranked first in malignant tumors, seriously threatening human health. Lung cancer is usually divided into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). The latter accounts for about 80% of all lung cancers [1]. Surgery and chemoradiotherapy are commonly used in the treatment of NSCLC [2]. Surgery is the preferred treatment approach of early NSCLC. However, due to the hidden onset of early NSCLC and atypical clinical symptoms of patients, patients would have already been in the medium and advanced stages at the time of diagnosis. Thus, they have lost the best opportunity for surgery and radical cure [3]. Therefore, chemotherapy has become the main treatment strategy of NSCLC. Chemotherapy, however, often leads to decreased immune function in patients, with severe toxic side effects [4].

Transforming growth factor (TGF) is an important inducer of epithelial-mesenchymal transition (EMT), of which TGF-α and TGF-β1 are closely related to invasion, metastasis, and prognosis of NSCLC [5]. Vascular endothelial growth factor (VEGF) is a kind of growth factor of vascular endothelial cells. It can promote the growth, invasion, and metastasis of NSCLC [6]. One of the most active ginsenosides, ginsenoside Rg3 has anti-tumor activity and immune function-regulating effects. It has been widely used in the treatment of various malignant tumors [7]. ^{125}I seed implantation is an *in vivo* radiotherapy, with advantages of low-dose continuous irradiation, accurate target, and short-term killing of tumor cells [8]. However, the

Table 1. Comparison of general materials between the two groups of patients

Item	Control group (n = 31)	Observation group (n = 31)	t/χ^2	p
Gender (male/female)	20/11	22/9	0.074	0.786
Age (years old)	45-75	45-80		
Average age (years old)	57.23 ± 6.59	56.89 ± 6.75	0.201	0.841
Pathological type [n (%)]				
Adenocarcinoma	21 (74.19)	23 (80.64)	0.325	0.850
Squamous carcinoma	6 (16.13)	5 (12.90)		
Alveolar carcinoma	4 (9.67)	3 (6.45)		

Table 2. Comparison of curative effects between the two groups of patients [n (%)]

Group	n	CR	PR	SD	PD
Observation group	31	12 (38.71)	9 (29.03)	6 (19.35)	4 (12.90)
Control group	31	6 (19.35)	7 (22.58)	7 (22.58)	11 (35.48)

Note: In rank sum test of curative effects in both groups of patients, Z = 2.300, p = 0.021.

Table 3. Comparison of body weights between the two groups of patients [n (%)]

Group	n	Increase	Stability	Decrease	Improvement rate
Observation group	31	11 (35.48)	15 (48.39)	5 (16.13)	26 (83.87)
Control group	31	6 (19.35)	8 (25.81)	17 (54.84)	14 (45.16)
χ^2		8.525			
p		0.004			

effects of ginsenoside Rg3 combined with ^{125}I seeds on serum TGF-α, TGF-β1, and VEGF in patients with NSCLC remain poorly understood. In this study, patients with medium and advanced NSCLC were treated with chemotherapy (gemcitabine-platinol (GP) regimen) combined with or without ginsenoside Rg3 + ^{125}I seed implantation. This study aimed to investigate the effects of ginsenoside Rg3 combined with ^{125}I seeds on serum levels of TGF-α, TGF-β1, and VEGF.

Materials and methods

General materials

A total of 62 patients with medium and advanced NSCLC, treated from June 2015 to May 2017, were selected and randomly divided into a control group (n = 31) and observation group (n = 31) using a random number table. Inclusion criteria: 1 Patients meeting the diagnostic criteria of NSCLC [9]; 2 Patients with expected survival times > 3 months; and 3 Patients providing informed consent. Exclusion criteria: 1 Patients complicated with severe hepatic or renal dysfunction or chemotherapy contraindications; 2 Patients accompanied with mental disorders; and 3 Patients seriously allergic to drugs used in this study. General materials showed no statistically significant differences between the two groups of patients ($p > 0.05$) (**Table 1**).

Methods

Treatment

GP regimen was used in the control group: gemcitabine (manufacturer: Eli Lilly and Company, USA, approval number: H20110535, 1250 mg/m^2, d1 & d8) + platinol (manufacturer: Yunnan BioValley Pharmaceutical Co., Ltd., approval number: NMPN H20043-888, 75 mg/m^2, d1). Based on the GP regimen, patients in the observation group were treated with ginsenoside Rg3 combined with ^{125}I seeds. Patients took Shenyi capsules orally (main ingredient: ginsenoside Rg3, manufacturer: Jilin Changchun Yatai Pharmaceutical Co., Ltd., approval number: NMPN Z20030043) before meals (20 mg/time, 2 times/day). According to preoperative computed tomography (CT) scans, the locations of tumors were determined. Depth, direction, and angles of the needle tip were adjusted using real-time CT scans to implant the ^{125}I seeds (Isotope Research Institute, Beijing Institute of Atomic Energy), using an implantation gun, until reaching the deepest tumor lesions at an interval of 0.5 cm.

Detection of indexes

Before treatment and at 4, 8, and 16 weeks after treatment, 5 mL venous blood was collected from patients (fasting for more than 8 hours) in both groups in the morning. It was

Table 4. Comparison of adverse reactions between the two groups of patients [n (%)]

Group	n	Gastrointestinal reaction	Abnormal liver function	Thrombocytopenia	Neutropenia	Total incidence rate of adverse reactions
Observation group	31	3 (9.68)	0 (0.00)	2 (6.54)	1 (3.23)	6 (19.35)
Control group	31	5 (11.13)	3 (9.68)	4 (12.90)	4 (12.90)	16 (51.61)
χ^2						5.707
p						0.017

centrifuged (centrifugal radius 15 cm) at 3000 rpm at 4°C for 10 minutes. The supernatant (serum) was collected to detect levels of TGF-α, TGF-β1, and VEGF via enzyme-linked immunosorbent assay (ELISA) kits, in strict accordance with manufacturer instructions. Relevant kits were provided by Beijing 4A Biotech Co., Ltd. Optical density (OD) values were measured at a wavelength of 450 nm using a microplate reader (Elx800, BIO-TEK) and levels of TGF-α, TGF-β1, and VEGF were calculated. After 2 courses of treatment, 3-5 mL fasting venous blood was collected from patients in both groups to isolate serum. Anti-human cluster of differentiation 3 (CD3), CD4, and CD8 antibodies were added into the serum, respectively, followed by incubation in the dark at 4°C for 30 minutes. T lymphocyte subsets (CD3+, CD4+, and CD8+ levels and CD4+/CD8+ ratios) were then detected by flow cytometry (BD, USA).

Evaluation criteria

Short-term curative effects were compared between the two groups using the therapeutic evaluation criteria of solid tumors as follows: Complete remission (CR), partial remission (PR), stable disease (SD), and progressive disease (PD). 1) CR: Disappearance of all visible lesions and maintenance time ≥ 4 weeks; 2) PR: Reduction of largest tumor diameter ≥ 50% and maintenance time ≥ 4 weeks; 3) SD: Non-CR and PR; 4) PD: Relative increase of target lesion diameter ≥ 20%, the increase of absolute value of diameter ≥ 5 mm, and emergence of new lesions. Objective response rate (ORR) = (CR + PR)/total cases, and disease control rate (DCR) = (CR + PR + SD)/total cases [10].

Changes in patient body weights during treatment were monitored and the judgment criteria were as follows: 1) Increase: Weight was increased ≥ 1 kg after treatment, compared with that before treatment, and it was maintained for more than 3 weeks; 2) Stability: Weight was increased or decreased < 1 kg after treatment, compared with that before treatment; 3) Weight was decreased ≥ 1 kg after treatment, compared with that before treatment. Improvement rate of weight = (increase + stability)/total cases. Adverse reactions, including thrombocytopenia, neutropenia, abnormal liver function, and gastrointestinal reactions, were compared between the two groups of patients.

Statistical analysis

Statistical Product and Service Solutions (SPSS) 19.0 (SPSS Inc., Chicago, IL, USA) software was used for data processing. Measurement data are presented as mean ± standard deviation (SD) and were analyzed by two-tailed unpaired Student's t-test. Rank sum tests were used to assess curative effects. Enumeration data are presented as percentages (%) and were compared by Chi-squared test. $P < 0.05$ suggests that differences are statistically significant.

Results

Comparison of curative effects

After 2 courses of treatment, ORR and DCR in the observation group (67.74% and 87.10%) were significantly higher than those in the control group (41.93% and 64.52%) ($p < 0.05$) (**Table 2**).

Comparison of body weights

After treatment, improvement rates of body weight in the observation group were significantly higher than those in the control group ($p < 0.05$) (**Table 3**).

Comparison of adverse reactions

The total incidence rate of adverse reactions in the observation group was significantly lower

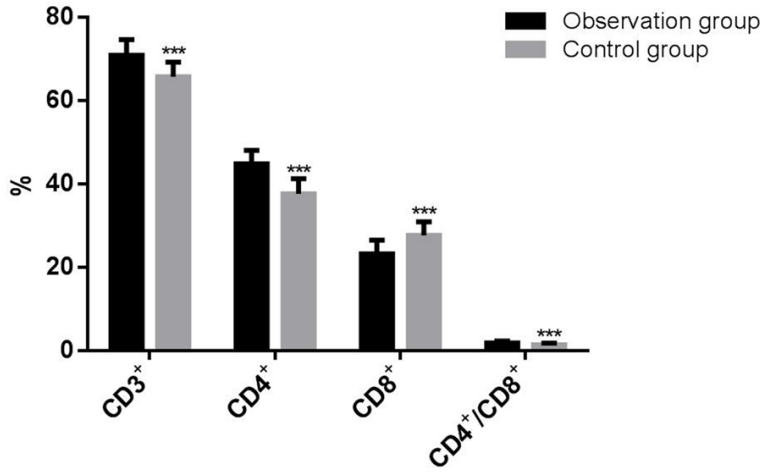

Figure 1. Comparison of levels of T lymphocyte subsets (%). Blood was collected from patients in the observation group and control group, followed by measuring CD3, CD4, and CD8 T lymphocytes (%) by flow cytometry. Compared with the observation group, ***p < 0.001.

than that in the control group (p > 0.05) (**Table 4**).

Comparison of levels of T lymphocyte subsets

After 2 courses of treatment, levels of CD3+ and CD4+ and CD4+/CD8+ ratios in the observation group were higher than those in the control group, but CD8+ levels were significantly lower than those in the control group (p < 0.05) (**Figure 1**).

Changes in serum levels of TGF-α, TGF-β1, and VEGF before and after treatment

At 4, 8, and 16 weeks after treatment, serum VEGF levels in patients in the two groups were significantly decreased, compared with those before treatment (p < 0.05), with more decreases seen in the observation group (**Figure 2A**). At 4, 8, and 16 weeks after treatment, levels of TGF-α and TGF-β1 in the control group were not changed significantly (p > 0.05), but they were significantly decreased in the observation group, compared with those before treatment (p < 0.05) (**Figure 2B** and **2C**).

Discussion

Lung cancer mostly originates from the bronchial mucous epithelium, also known as bronchopulmonary carcinoma. There are many incentives of NSCLC, such as occupational carcinogens, air pollution, smoking, diet and nutrition, ionizing radiation, and genetic changes [11]. The pathogenesis of NSCLC is not yet fully understood, but it is generally believed that invasion of carcinogenic factors into the lungs leads to local cell damage under the influence of various incentives. Immune mechanisms of the body begin to remove carcinogenic factors and the lungs initiate self-healing effects. When the carcinogenic factors cannot be completely removed, local inflammation will occur, creating a harsh microenvironment. Thus, it is difficult for lung tissue cells to adapt to the environment, leading to occurrence of malignant proliferation over time and subsequent development of NSCLC [12].

Ginsenoside Rg3, namely tetracyclic triterpene saponin, is the most biologically active steroid compound isolated from ginseng, the monomeric saponin with anti-tumor effects [13]. Related studies have shown that [14] ginsenoside Rg3 can reduce proliferation and inhibit the growth of tumor cells, promote tumor cell apoptosis, and inhibit invasion and metastasis of cancer cells. With the continuous development of radiobiology and physics, radioactive seed implantation has been increasingly applied in the treatment of solid tumors. Commonly-used radionuclide sources include cesium-137, iodine-125, and iridium-192 [15]. In the present study, the observation group displayed significantly higher ORR and DCR, improved body weight changes, and lower overall incidence rates of adverse reactions, compared with the control group (p < 0.05). This is because ginsenoside Rg3 can affect the cycle of lung tumor cells and slow down the proliferation of tumor cells. Thus, it exerts anti-tumor effects. Moreover, ^{125}I seeds can reduce tumor volume through continuous superposition more easily, compared with chemotherapy alone. The combination of them can enhance the effects of chemotherapy. Additionally, ^{125}I seeds are constant in the target area and will not be changed along with the movement of irradiation site without causing damage to the sur-

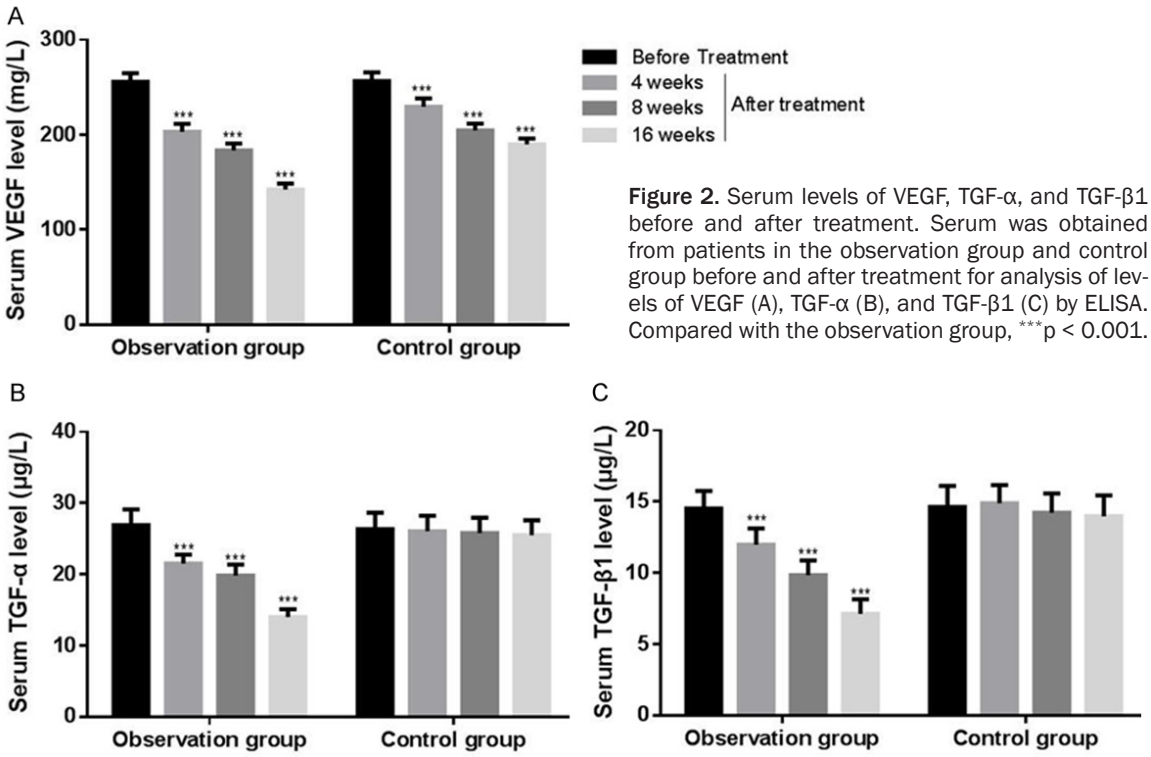

Figure 2. Serum levels of VEGF, TGF-α, and TGF-β1 before and after treatment. Serum was obtained from patients in the observation group and control group before and after treatment for analysis of levels of VEGF (A), TGF-α (B), and TGF-β1 (C) by ELISA. Compared with the observation group, $^{***}p < 0.001$.

rounding normal tissue cells. This does not increase the toxic side effects but can increase the sensitivity of tumor cells to chemoradiotherapy. These ^{125}I seeds, combined with ginsenoside Rg3, can reduce the toxic side effects of chemotherapy, thereby improving abnormal changes in body weights of patients.

T lymphocytes participate in the immune response of the body and regulate immune function. Of these, CD3+ represents the total number of T lymphocytes in the body and CD4+ is a helper T lymphocyte, which enhances regulatory effects on immune response. CD8+ is a suppressor T lymphocyte, which can destroy and kill infected cells. It is associated with several immune function disorders [16, 17]. Present results show that levels of CD3+ and CD4+ and CD4+/CD8+ ratios in the observation group, after 2 courses of treatment, were significantly higher than those in control group, but levels of CD8+ were significantly lower than those in the control group ($p < 0.05$). This is because ginsenoside Rg3, an important extract of ginseng, can enhance immunity, increase the number of immune cells involved in immune response and immune activity, increase the number of CD3+ and CD4+, regulate the immune balance status, and improve the ability of the body to remove the virus. When CD4+/CD8+ ratios are increased, immune function will be enhanced. Its ability to clear the virus will also be enhanced.

VEGF is a member of the platelet-derived growth factor family. It is currently an angiogenic factor with stronger activity and highest specificity. It stimulates vascular endothelial cells and promotes their division and proliferation, eventually leading to neovascularization. In tissues and serum of many tumor patients, VEGF is highly expressed, leading to increased proliferation of tumor cells and subsequent lymphatic and hematogenous metastases [18, 19]. Under conditions of overexpression of TGF-α and TGF-β, the development of medium and advanced tumors will be promoted. TGF-β1 is an immunomodulatory factor widely involved in various physiological and pathological processes in the body, playing dual roles in cells. It inhibits the proliferation of normal cells, while promoting cell invasion and metastasis in m-a lignant tumors in advanced stages [20]. This study showed that at 4, 8, and 16 weeks after treatment, serum VEGF levels in patients in the two groups were significantly decreased, com-

pared with those before treatment, with more significant decreases seen in the observation group. Moreover, levels of TGF-α and TGF-β1 in the control group were not changed significantly, but they were significantly decreased in the observation group, compared with those before treatment ($p < 0.05$). Moreover, ^{125}I seeds can release characteristic electrons and photons, controlling and killing the tumor cells in the target region. These ^{125}I seeds, combined with ginsenoside Rg3, can downregulate expression of MMPs, regulate TGF-β1 signaling pathways, reduce expression of TGF-β1, and downregulate expression of TGF-α, thus reversing EMT. The seeds exert inhibiting effects on metastasis of tumor cells in middle and advanced stages. They also slow down cell division rates and inhibit tumor cell growth.

Conclusion

In conclusion, ginsenoside Rg3, combined with ^{125}I seed implantation, can improve patient immunity, reduce toxicity, and enhance efficacy of routine chemotherapy, as well as inhibit TGF-α, TGF-β1, and VEGF expression.

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Disclosure of conflict of interest

None.

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