

## **Combinational effect of endostar bolus infusion and chemotherapy in small cell lung cancer.**

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### **Abstract**

**Objective:** To investigate the combinational effect of Endostar bolus infusion and chemotherapy on the level of serum Vessel Epithelium Growth Factor (VEGF), circulating endothelial cells and endostatin in small cell lung cancer patients.

**Materials and methods:** From January, 2011 to December, 2014, 30 patients who were diagnosed as III-IV stage of small cell lung cancer were referred to our hospital. All patients were divided randomly and evenly into three groups: chemotherapy group (Group A), Endostar conventional infusion/chemotherapy group (Group B) and Endostar bolus infusion/chemotherapy group (Group C). The level of VEGF, circulating endothelial cells and endostatin in serum of all patients were detected by Enzyme-Linked Immunosorbent Assay (ELISA).

**Results:** After 4 w of treatment, the endostatin level of patients in group C increased, while the level of VEGF and circulating endothelial cells in their serum decreased. But there was no significance found when compared with patients in groups A and B ( $P>0.05$ ). However, after 8 and 12 w of treatment, the difference between group C and groups A and B became significant ( $P<0.05$ ).

**Conclusion:** This study revealed that combination of Endostar bolus infusion and chemotherapy can increase the endostatin level, and decrease the level of VEGF and circulation endothelial cells in serum of small cell lung cancer patients, which suggests that the combination of endostar bolus infusion and chemotherapy can inhibit angiogenesis in small lung cancer.

**Keywords:** Endostar bolus infusion, Small cell lung cancer, VEGF, Circulating endothelial cells, Endostatin.

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### **Introduction**

Malignant tumor is one of the most prevalent and lethal diseases that threaten health of human beings in the world [1]. The traditional strategies always used for treatment of cancer are combination of surgery and chemotherapy, however, the effect of these strategies is not as good as we expect. Hence, new strategies are urgently. As one of the most favourite targets, angiogenesis is every pivotal in carcinogenesis and metastasis [2]. Angiogenesis is a complicated process which is regulated by many factors, including Vascular Endothelial Growth Factor (VEGF), Fibroblast Growth Factor (FGF), angiogenin, Epidermal Growth Factor (EGF) and Tumor Necrosis Factor (TNF) [3-5]. Endostatin exists in almost all tissues in our body, especially in the wall of blood vessels and the matrix membrane. Endostatin can inhibit angiogenesis through repressing the proliferation and migration of endothelial cells, which will cut off the supplement of nutrition

to cancer cells [6]. Circulating endothelial cells are the endothelial cells detected in the circulation. The emergence of circulating endothelial cells in circulation is mainly caused by endothelium damage. Besides, circulating endothelial cells is now the main indicator of endothelium damage in living body [7]. Endostar is the injection consists of recombinant human endostatin which is developed by the Chinese scientist and has been approved to go on the market. The mechanism of endostar is inhibiting carcinogenesis through cutting off the nutrition supplement to cancer cells [8]. Our study investigated the effect of combination of Endostar infusion and chemotherapy on the level of VEGF, circulating endothelial cells and endostatin in serum of patients suffering small cell lung cancer, which provides theoretical evidence to treatment of small cell lung cancer with endostar.

## Materials and Methods

### Patients

From January, 2011 to December, 2014, 30 patients who were diagnosed as III-IV stage of small cell lung cancer were referred to the department of oncology in Dongying people's hospital, Shandong. All patients were divided randomly and evenly into three groups: chemotherapy group (A), Endostar conventional infusion/chemotherapy group (B) and Endostar bolus infusion/chemotherapy group (C). All patients accepted the treatment first time and tumors were detected in their bodies by CT or ultrasonic test. No patients in the study were suffering liver diseases, kidney diseases, or heart diseases.

### Ethical approval

This study is conducted accord to the standards of Good Clinical Practice and in compliance with local regulations. Oral and written informed consent forms are obtained from all patients prior to treatment.

### Endostar infusion

Endostar conventional infusion is performed as intravenous infusion with 7.5 mg/m<sup>2</sup> for 4 h; Endostar bolus infusion is conducted as fast intravenous infusion of 15mg, followed by a continuous intravenous infusion of 150 mg for 24 h. The drugs used to stop vomiting response are provided.

### ELISA

The levels of VEGF and endostatin in serum were determined by ELISA (Enzyme-linked Immunosorbent Assay) using a specific kit produced by the BlueGenes Biotech company (Shangai, China) with an analytic sensitivity of 0.1 ng/mL according to the manufacturer's instructions. Readings were generated using a BioTek Absorbance Reader, ELX800 model (Winooski, VT, USA).

### Follow-up study

To check the long-term effect and side effect of Endostar bolus infusion, the follow-up study is carried out every 4-8 w. In the follow-up study, we take the Eastern Cooperative Oncology Group (ECOG) score, imaging examination and clinical lab test to check the situation of all participants.

### Statistical analysis

Statistical analysis is performed with SPSS 10.0 software. Data is presented as mean  $\pm$  Standard Deviation (SD). The difference between different groups was evaluated through t test and ANOVA. A value of  $P < 0.05$  is considered as statistically significant.

## Results

### The general information of all participants

From January, 2011 to December, 2014, 30 patients, who were diagnosed as suffering stages III-IV small cell lung cancer, were referred to our department. To compare the effect of different treatment strategies, the patients were divided into three groups: chemotherapy group (Group A), Endostar conventional infusion/chemotherapy group (Group B) and Endostar bolus infusion/chemotherapy group (Group C). The general information of all participants showed that there was no difference in age, sex, tumor stage and metastasis of patients in three groups (Table 1).

**Table 1.** General information of all related patients.

	Group (n=10)	A Group (n=10)	B Group (n=10)	C t	P
Age	75.2 $\pm$ 7.1	74.5 $\pm$ 6.5	75.5 $\pm$ 5.5	2.12	0.325
Male	5	6	5	3.24	0.578
Female	5	4	5	1.95	0.545
Stage IIIA/IIIB tumor	2	3	3	2.47	0.254
Stage IV tumor	8	7	7	2.23	0.317
Lymphoma metastasis	5	5	5	3.11	0.511
Liver metastasis	8	7	8	2.21	0.436

### Comparison of serum VEGF level of all patients in all groups

Even though there was no significant difference in serum VEGF level among all three groups before treatment, after 4 w of treatment, the serum VEGF level of patients in group C became lower than those in group A and B. Next, after 8 w and 12 w of treatment, the VEGF level in serum of patients in group C became much lower than those in groups A and B (Table 2).

### Comparison of serum endostatin level of all patients in all groups

Before treatment, there was no significant difference in serum level of endostatin among all three groups. However, after 4 w, 8 w and 12 w of treatment, the endostatin level of patients in groups B and C increased obviously, and much higher than those in group A. No significant change of endostatin level in serum of patients in group A was found (Table 3).

### Comparison of circulating endothelial cells in serum of all grouped patients

Before the treatment, there was no difference in number of endothelial cells in circulation of patients in all groups. In 4 w of treatment, the number of endothelial cells in patients from

group C decreased, but no significant difference was identified when compared with the number of circulating endothelial cells of patients in groups A and B. However, after 8 and 12 w

of treatment, the number of endothelial cells in patients from group C is much less than those of patients in groups A and B, the difference is significant (Table 4).

**Table 2.** Comparison of serum VEGF level of all patients in all groups.

Time	n	VEGF (pg/ml)			t	P
		Group A	Group B	Group C		
Pre-treatment	10	75.13 ± 4.49	74.34 ± 10.55	78.95 ± 4.73	2.32	0.239
4 w Post-treatment	10	69.26 ± 6.09	69.45 ± 6.45	53.28 ± 6.42	3.11	0.051
8 w Post-treatment	10	65.13 ± 4.50	64.26 ± 6.10	38.95 ± 4.73*	3.01	0.039
12 w Post-treatment	10	64.96 ± 5.69	59.25 ± 6.05	31.70 ± 5.96*	3.17	0.015

\*P<0.05, the difference among groups A-C is significant.

**Table 3.** The level of endostatin in serum of all patients.

Time	N	Endostatin (µmol/L)			t	P1	P2
		Group A	Group B	Group C			
Pre-treatment	10	15.01 ± 5.45	14.34 ± 1.55	18.05 ± 4.73	2.578	0.159	0.113
4 w post-treatment	10	19.26 ± 6.09	29.45 ± 6.45	43.28 ± 6.42*	1.932	0.021	0.057
8 w post-treatment	10	15.13 ± 4.49	29.26 ± 6.09	48.95 ± 4.73*#	1.665	0.016	0.044
12 w post-treatment	10	14.96 ± 5.69	31.35 ± 5.05	58.70 ± 5.96*#	2.031	0.004	0.025

\*P1<0.05, the difference between groups A and C is significant; #P2<0.05, the difference between B and C is significant.

**Table 4.** The number of circulating endothelial cells in serum of all patients.

Time	n	Circulating endothelial cells (cells/µL)			t	P
		Group A	Group B	Group C		
Pre-treatment	10	119.01 ± 11.45	119.34 ± 16.55	118.05 ± 17.73	2.97	0.203
4 w post-treatment	10	116.16 ± 14.05	120.45 ± 12.45	115.37 ± 11.22	3.01	0.219
8 w post-treatment	10	115.68 ± 12.50	116.26 ± 11.27	69.98 ± 13.26*	5.22	0.037
12 w post-treatment	10	110.15 ± 12.57	108.36 ± 16.42	54.51 ± 11.92*	5.47	0.013

\*P<0.05, the difference among groups A-C is significant.

## Discussion

Malignant tumor is a kind of systematic disease caused by many different factors. As one of the most important factors that facilitate development of cancer, angiogenesis provides the nutritional foundation to cancer cells. Thus, the treatments aiming at inhibition of angiogenesis attract more and more attention. The growth of tumor relies on angiogenesis. The formation of new blood vessels not only provides more nutrition to tumor tissue, but also facilitates the metastasis through delivering cancer cells from primary location to target organs. The process of angiogenesis is so complicated and under the control of many different factors, such as vascular endothelial growth factor, fibroblast growth factor, angiogenin,

epidermal growth factor, IL-8 and tumor necrosis factor [3-5]. In 1997, O'Reilly and Folkman [9] found the existence of endostatin in different tissues of human body, especially in the wall of blood vessels and the matrix membrane area. Endostatin is a factor which can inhibit angiogenesis through suppressing the proliferation and migration of endothelial cells, which has been approved both *in vitro* and *in vivo* [10,11]. The mechanism of how endostatin influence the proliferation and migration of endothelial cells is still not crystal clear. But the most popular hypothesis is that endostatin can affect the binding of VEGF with its receptor. Moreover, other evidence showed that endostatin also resulted in down-regulation of VEGF in both mRNA and protein level [12]. Hence, endostatin

can block the signaling pathway mediated by VEGF and suppress angiogenesis.

Circulating endothelial cells refers to endothelial cells detected in circulation. The number of circulating endothelial cells indicates the damage of endothelium. As the only indicator of vascular damage in live body, endothelial cells accumulate in circulation when inflammation, infection, tumor, and heart disease happen [13]. Francesco et al. [14] found that the number of endothelial cells in circulation of patients suffering cancer was much more than those of healthy people.

Endostar is a kind of intravenous infusion containing recombinant human endostatin. In 2006, Endostar was approved by Chinese National Cancer Center to be used in treatment of non-small cell lung cancer. The Endostar can inhibit carcinogenesis and metastasis through suppressing the proliferation and migration of endothelial cells that are important to angiogenesis [15-17].

In our study, we found that combination of Endostar bolus infusion and chemotherapy resulted in obvious decrease of VEGF and circulating endothelial cells in the serum of patients who suffered small cell lung cancer. In addition, even though traditional Endostar infusion can also increase the endostatin level in serum, Endostar bolus infusion showed much better effect. Hence, our data implies that combination of chemotherapy with Endostar bolus infusion is a good strategy to treat small cell lung cancer and worth the application in clinic.

## Conclusion

Combination of endostar bolus infusion and chemotherapy can increase the endostatin level, and decrease the level of VEGF and circulation endothelial cells in serum of small cell lung cancer patients, which suggests that the combination of Endostar bolus infusion and chemotherapy can inhibit angiogenesis in small lung cancer.

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