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Clinical study on bevacizumab combined with carboplatin therapy for malignant pleural effusion of non−small cell lung cancer

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ABSTRACT

Objective: To investigate the effect of bevacizumab combined with carboplatin therapy for malignant pleural effusion of non-small cell lung cancer on tumor markers, angiogenesis molecules and invasive growth molecules. **Methods:** A total of 68 patients who were diagnosed with non-small cell lung cancer complicated by pleural effusion in the Affiliated T.C.M Hospital of Southwest Medical University between June 2013 and August 2016 were selected and randomly divided into two groups, the combined group received bevacizumab combined with carboplatin chemotherapy, and the carboplatin group received carboplatin chemotherapy. Before treatment as well as 3 cycles and 6 cycles after treatment, the contents of tumor markers, angiogenesis molecules and invasive growth molecules in pleural effusion were examined. **Results:** 3 cycles and 6 cycles after treatment, CEA, SCCAg, CYFRA21-1, sHLA-G, VEGF, VEGFR, PTN, MMP7 and MMP10 contents in pleural effusion of both groups of patients were significantly lower than those before treatment while TIMP1 and TIMP2 contents were significantly higher than those before treatment, and CEA, SCCAg, CYFRA21-1, sHLA-G, VEGF, VEGFR, PTN, MMP7 and MMP10 contents in pleural effusion of combined group were significantly lower than those of carboplatin group while TIMP1 and TIMP2 contents were significantly higher than those of carboplatin group. **Conclusion:** Bevacizumab combined with carboplatin therapy for malignant pleural effusion of non-small cell lung cancer can effectively kill cancer cells, and inhibit angiogenesis and cell invasion.

1. Introduction

Non-small cell lung cancer (NSCLC) is a clinical common malignant tumor, it is without early specific clinical symptoms and with low early diagnostic rate, it has developed to middle-advanced stage in majority of patients at diagnosis and the cancer cells show invasive growth. Malignant pleural effusion is a common complication of advanced NSCLC, the occurrence of pleural effusion is associated with the cancer cell infiltration to the pleura, and the chest drainage and local chemotherapy drug injection could delay the disease progression to a certain extent. Platinum-based chemotherapy drugs are commonly used to treat malignant pleural effusions, they can effectively kill cancer cells after injected into the chest cavity, but the overall effect is limited[1]. Bevacizumab is a targeted drug to cure malignant tumor in recent years, which can be combined with vascular endothelial growth factor (VEGF) to inhibit its biological effect on promoting angiogenesis and then inhibit the growth and infiltration of cancer cells[2,3]. In the following study, the effectiveness of bevacizumab combined with carboplatin in treating malignant pleural effusion of non-small cell lung cancer was analyzed.

2. Information and methods

2.1 Case information

Prospective randomized single-center controlled study was designed, 68 patients who were diagnosed with non-small cell lung cancer complicated by pleural effusion in the Affiliated T.C.M Hospital of Southwest Medical University between June

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2013 and August 2016 were selected, all patients were diagnosed with advanced non-small cell lung cancer by the pathology biopsy, ultrasound confirmed that they were complicated by pleural effusion, and the expected survival time was > 3 months. Random number table was used to divide the 68 patients into two groups, 34 in each group. Combined group of patients received bevacizumab combined with carboplatin chemotherapy, including 22 male cases and 12 female cases that were 53-67 years old; carboplatin group received carboplatin chemotherapy, including 23 male cases and 11 female cases that were 51-65 years old. There was no significant difference between the two groups of patients (P>0.05).

2.2 Chemotherapy

After admission, both groups of patients received systemic chemotherapy, thoracocentesis and catheter indwelling, and the pleural effusion was extracted and continuously drained, which lasted for consecutive 2-3 d. The carboplatin group received intrapleural injection of carboplatin 50 mg/kg, once every 3 weeks, for a total of 2 times; combined group received intrapleural injection of bevacizumab combined with carboplatin, the bevacizumab dose was 5 mg/kg, the carboplatin dose was the same as that of carboplatin group, and treatment was done once every three weeks, for a total of 2 times.

2.3 Malignant degree evaluation

Before treatment as well as 3 cycles and 6 cycles after treatment, pleural drainage fluid samples were collected from two groups of patients respectively and centrifuged, the cell residue was abandoned, the supernatant was kept, and then the enzyme-linked immunosorbent assay kits were used to determine CEA, SCCAg, CYFRA21-1, sHLA-G, VEGF, VEGFR, PTN, MMP7, MMP10, TIMP1 and TIMP2 contents.

2.4 Statistical methods

SPSS 16.0 software was used to the detection indexes in pleural effusion, analysis of above indexes between two groups was by t test and P<0.05 indicated statistical significance in differences.

3. Results

3.1 Tumor marker contents in pleural effusion

Before treatment as well as 3 cycles and 6 cycles after treatment, analysis of tumor markers CEA (μg/L), SCCAg (ng/L), CYFRA21-1 (μg/L) and sHLA-G contents in pleural effusion between two groups of patients was as follows: (1) before treatment, CEA, SCCAg, CYFRA21-1 and sHLA-G contents in pleural effusion were not significantly different between two groups of patients (P>0.05); (2) 3 cycles and 6 cycles after treatment, CEA, SCCAg, CYFRA21-1 and sHLA-G contents in pleural effusion of both groups of patients were significantly lower than those before treatment (P<0.05); (3) CEA, SCCAg, CYFRA21-1 and sHLA-G contents in pleural effusion were significantly significant between two groups of patients 3 cycles and 6 cycles after treatment (P<0.05).

3.2 Angiogenesis molecule contents in pleural effusion

Before treatment as well as 3 cycles and 6 cycles after treatment, analysis of angiogenesis molecules VEGF (μg/L), VEGFR (ng/L) and PTN (ng/L) contents in pleural effusion between two groups of patients was as follows: (1) before treatment, VEGF, VEGFR and PTN contents in pleural effusion were not significantly different between two groups of patients (P>0.05); (2) 3 cycles and 6 cycles after treatment, VEGF, VEGFR and PTN contents in pleural effusion of both groups of patients were significantly lower than those before treatment (P<0.05); (3) VEGF, VEGFR and PTN contents in pleural effusion were significantly significant between two groups of patients 3 cycles and 6 cycles after treatment (P<0.05).

Table 1.

Tumor marker contents in pleural effusion of two groups of patients before and after treatment.

Groups	n	Time points	CEA	SCCAg	CYFRA21-1	sHLA-G
Combined group	34	Before treatment	203.94±31.25	42.38±5.14	35.93±5.62	31.28±5.28
		3 cycles after treatment	98.35±11.25*#	17.58±2.63*#	12.31±1.64*#	13.21±2.32*#
		6 cycles after treatment	64.21±8.93*#	11.28±1.85*#	9.39±1.03*#	8.58±1.14*#
Carboplatin group	34	Before treatment	206.41±28.96	44.03±6.58	36.39±5.27	30.89±5.68
		3 cycles after treatment	142.32±17.97*	24.21±3.85*	20.31±3.86*	19.49±2.62*
		6 cycles after treatment	115.27±13.47*	18.58±2.32*	14.48±1.93*	12.58±1.93*

*: comparison between before and after treatment, P<0.05; #: comparison between combined group and carboplatin group, P<0.05.

Table 2.

Angiogenesis molecule contents in pleural effusion of two groups of patients before and after treatment.

Groups	n	Time points	VEGF	VEGFR	PTN
Combined group	34	Before treatment	14.29±2.03	326.78±52.62	132.58±17.95
		3 cycles after treatment	7.31±0.93*#	146.24±18.65*#	64.39±8.12*#
		6 cycles after treatment	4.42±0.79*#	98.31±11.26*#	36.42±5.62*#
Carboplatin group	34	Before treatment	14.88±2.26	332.12±49.58	134.11±18.12
		3 cycles after treatment	10.27±1.85*	203.59±33.18*	94.51±11.25*
		6 cycles after treatment	7.12±0.94*	163.82±21.32*	59.63±8.32*

*: comparison between before and after treatment, P<0.05; #: comparison between combined group and carboplatin group, P<0.05.

Table 3.

Invasive growth molecule contents in pleural effusion of two groups of patients before and after treatment.

Groups	*n*	Time points	MMP7	MMP10	TIMP1	TIMP2
Combined group	34	Before treatment	5.94±0.89	3.28±0.51	223.55±32.52	168.61±20.34
		3 cycles after treatment	2.31±0.35*#	1.78±0.23*#	485.96±64.92*#	302.14±42.32*#
		6 cycles after treatment	1.57±0.23*#	1.03±0.14*#	641.29±79.66*#	426.96±55.87*#
Carboplatin group	34	Before treatment	6.03±0.81	3.35±0.58	226.12±29.37	170.12±23.52
		3 cycles after treatment	3.88±0.59*	2.44±0.32*	335.68±41.29*	244.52±32.86*
		6 cycles after treatment	2.52±0.37*	1.92±0.25*	409.59±56.41*	302.94±46.29*

*: comparison between before and after treatment, $P<0.05$; #: comparison between combined group and carboplatin group, $P<0.05$.

3.3 Invasive growth molecules in pleural effusion

Before treatment as well as 3 cycles and 6 cycles after treatment, analysis of invasive growth molecules MMP7 (μg/L), MMP10 (μg/L), TIMP1 (ng/L) and TIMP2 (ng/L) contents in pleural effusion between two groups of patients was as follows: (1) before treatment, MMP7, MMP10, TIMP1 and TIMP2 contents in pleural effusion were not significantly different between two groups of patients ($P>0.05$); (2) 3 cycles and 6 cycles after treatment, MMP7 and MMP10 contents in pleural effusion of both groups of patients were significantly lower than those before treatment while TIMP1 and TIMP2 contents were significantly higher than those before treatment ($P<0.05$); (3) MMP7, MMP10, TIMP1 and TIMP2 contents in pleural effusion were significantly significant between two groups of patients 3 cycles and 6 cycles after treatment ($P<0.05$).

4. Discussion

The cancer cell proliferation and invasion to the pleura is the key biological behavior to cause pleural effusion in patients with advanced NSCLC, the local application of chemotherapy drug carboplatin can form a relatively high drug concentration in the chest cavity and kill cancer cells, and it is widely used in the treatment of malignant pleural effusion. Bevacizumab is the humanized monoclonal antibody of VEGF, which can target and be combined with VEGF and inhibit its biological effects on promoting angiogenesis and cell growth[4,5]. In the study, bevacizumab combined with carboplatin was used to treat malignant pleural effusion, and in order to specify the therapeutic value of the combined medication, the contents of tumor markers in the pleural effusion were analyzed. In the process of infiltrating to pleura and forming pleural effusion, the cancer cells can synthesize the CEA, SCCAg, CYFRA21-1, sHLA-G and other markers and secrete them into the pleural effusion. CEA is a tumor antigen associated with a variety of malignant tumors, SCCAg is specific antigen of the squamous epithelial cells and its generation increases during the malignant transformation of squamous epithelial cells, and CYFRA21-1 is cytokeratin 19 fragment and is massive secreted into the outside of cells during the malignant change of epithelial cells[6,7]; HLA-G is a type of tumor-related immune response molecule, and it becomes sHLA-G after falling off into the blood circulation or the pleural effusion[8]. In the study, comparison of the levels of these tumor markers in pleural effusion between two groups of patients before and after treatment showed that CEA, SCCAg, CYFRA21-1 and sHLA-G contents in pleural effusion of both groups of patients after treatment were significantly lower than those before treatment, and CEA, SCCAg, CYFRA21-1 and sHLA-G contents in pleural effusion of combined group after treatment were significantly lower than those of carboplatin group. This means that both carboplatin monotherapy and bevacizumab combined with carboplatin can effectively kill the cancer cells infiltrating in pleural cavity, and the combination therapy has better killing effect on cancer cells than monotherapy.

Angiogenesis is an important biological feature in the progression of malignant tumors, and also an important cause of malignant pleural effusion formation. The new blood vessel structure is characterized by high permeability, and plasma protein is easily permeable and affects the internal and external pleural osmotic pressure, which is beneficial to the formation of pleural effusion. In addition, the newborn blood vessels can on the one hand, provide nutrition for the proliferation of cancer cells, and on the other hand, provide pathways for the infiltration of cancer cells, which is advantageous to the cancer cell infiltration to the pleura and promotes the formation of pleural effusion[9]. VEGF is the most important cytokine mediateing angiogenesis, it is also the target of bevacizumab, the locally synthesized and secreted VEGF is combined with receptor VEGFR to exert the biological effects on promoting the endothelial cell growth and promoting blood vessel structure formation, and it is conducive to the formation of new blood vessels[10,11]. PTN is a newly discovered cancer cell marker protein with transformant characteristics in recent years, which can induce endothelial cell transformation and proliferation, and create favorable local environment for angiogenesis[12,13]. In order to define the effect of bevacizumab plus carboplatin on angiogenesis in local lesion of NSCLC patients with malignant pleural effusion, the levels of these angiogenesis molecules in pleural effusion were compared between two groups of patients before and after treatment in the study, and it was found that VEGF, VEGFR and PTN contents in pleural effusion of both groups of patients after treatment were significantly lower than those before treatment, and VEGF, VEGFR and PTN contents in pleural effusion of combined group after treatment were significantly lower than those of control group.

This means that both carboplatin monotherapy and bevacizumab combined with carboplatin can effectively inhibit angiogenesis process, and the combination therapy has better inhibiting effect on angiogenesis than monotherapy.

The invasive growth of cancer cells from primary lung cancer lesions to the pleura is the important cause of the formation of pleural effusion, and a variety of molecules in matrix metalloproteinase family mediate the invasive growth of cancer cells. MMP7 and MMP10 are the matrix metalloproteinases closely related to the cancer cells invasion, and they can not only directly degrade a variety of ingredients in extracellular matrix, but can also degrade E-cadherin and reduce the degree of intercellular adhesion, which is advantageous to the cell movement and invasion[14,15]. The abnormal high expression of MMP7 and MMP10 in lung cancer is beneficial for the invasion of lung cancer cells to the pleura as well as the formation of malignant pleural effusion[16]. TIMP1 and TIMP2 are the specific suppressors of matrix metalloproteinase, which can inhibit the catalytic activity of MMP7 and MMP10 so as to inhibit the cell invasion[17]. In order to define the effect of bevacizumab plus carboplatin on the invasive growth of cells in local lesion of NSCLC patients with malignant pleural effusion, the levels of these invasive growth molecules in pleural effusion were compared between two groups of patients before and after treatment in the study, and it was found that MMP7 and MMP10 contents in pleural effusion of both groups of patients after treatment were significantly lower than those before treatment while TIMP1 and TIMP2 contents were significantly higher than those before treatment, and MMP7 and MMP10 contents in pleural effusion of combined group after treatment were significantly lower than those of control group while TIMP1 and TIMP2 contents were significantly higher than those of control group. This means that both carboplatin monotherapy and bevacizumab combined with carboplatin can effectively inhibit the invasive cell growth, and the combination therapy has better inhibiting effect on invasive cell growth than monotherapy.

To sum up, it is believed that bevacizumab plus carboplatin is more valuable than carboplatin monotherapy in the treatment of non-small cell lung cancer with malignant pleural effusion, and the combination therapy can more effectively kill the cancer cells, and inhibit angiogenesis and cell invasion.

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