Intrapleural combination therapy with bevacizumab and cisplatin for non-small cell lung cancer-mediated malignant pleural effusion

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Abstract. Malignant pleural effusion (MPE) is a common complication of advanced non-small cell lung cancer (NSCLC). Bevacizumab, a humanized monoclonal antibody against vascular endothelial growth factor (VEGF), has been shown to be efficient in suppressing the accumulation of pleural fluid. However, whether intrapleural delivery of bevacizumab can be used to treat MPE remains unknown. The aim of the present study was to evaluate the efficacy and safety of combined intrapleural therapy with bevacizumab and cisplatin, an antineoplastic agent, in controlling MPE. A total of 72 NSCLC study subjects with MPE were randomly assigned to one of two groups. The first group received intrapleural bevacizumab (300 mg) with cisplatin (30 mg) therapy and the second group received intrapleural cisplatin (30 mg) therapy alone. Pleural fluid was collected from both groups prior to and following treatment. The levels of VEGF and carcinoembryonic antigen (CEA) in the pleural fluid were determined by ELISA. In 70 evaluable study subjects, the curative efficacy in the bevacizumab group was significantly higher than that found in the cisplatin group (83.33 vs. 50.00%, respectively; p<0.05). Therapy with combined bevacizumab plus cisplatin significantly reduced VEGF levels in the pleural fluid (p<0.01). In the bevacizumab group, the levels of VEGF in the pleural fluid were significantly lower compared to those of the cisplatin group after treatment, which showed greater efficacy (p<0.01). In addition, combination therapy showed greater efficacy in the patients with high levels of VEGF expression (p<0.01). There was no significant difference in grade III/IV adverse events between the two groups. All procedures were well tolerated by the patients. Combined intrapleural therapy with bevacizumab and cisplatin was effective and safe in managing NSCLC-mediated MPE. We propose that VEGF expression levels in MPE could serve as a prognostic marker for bevacizumab therapy.

Introduction

Malignant pleural effusion (MPE) is a common complication of advanced metastatic non-small cell lung cancer (NSCLC). Abnormal fluid accumulation in the pleural space could lead to dyspnoea, cough and chest pain, which may eventually impair the normal function of the heart, and thus threaten patient survival (1). Since MPE presents with a high incidence and poor prognosis, characterized by a medium survival rate of 3 months, one of the highest priorities is to actively manage the pleural effusion in an attempt to improve the quality of life of the patients (1,2). The current mode of therapy for patients with MPE primarily depends on local treatment, such as tube drainage, chemical pleurodesis and intrapleural administration of antineoplastic agents such as doxorubicin, cisplatin, cytarabine, carboplatin, etoposide, mitomycin C and 5-fluorouracil (3-6). Of these chemotherapeutic agents, cisplatin is the most commonly used for the treatment of NSCLC. It is thus the most frequently used drug for intrapleural therapy of pleural effusions caused by NSCLC (7). Although the current mode of therapy can occasionally alleviate the symptoms, the relapse rate is as high as 50%. In addition, for most patients, no single treatment approach can achieve a satisfactory effect. Consequently, there is an urgent need for highly effective combinatorial therapies.

Vascular endothelial growth factor (VEGF) is an endothelial cell-specific growth factor that stimulates vasculogenesis and angiogenesis, and has been shown to be an essential intermediate in the formation of pleural effusions (8-10). Synthesis of VEGF by both autocrine and paracrine pathways (11) has shown it to be present in multiple solid cancers including NSCLC. Overexpression of VEGF in NSCLC can enhance its interaction with VEGF-specific receptors. Occupation of these receptors by VEGF could further stimulate the production of VEGF by both cancer and stroma cells, leading to local increases in the levels of VEGF in MPE.

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It is well documented that bevacizumab, a humanized monoclonal antibody raised against VEGF, is able to directly inhibit proliferation, migration and differentiation of vascular endothelial cells. Bevacizumab can also promote apoptosis of endothelial cells and suppress VEGF-induced neoangiogenesis and vascular permeability (12). Of note, bevacizumab has been shown to synergize with chemotherapeutic agents to block the accumulation of pleural fluid (13-17), thus making it a promising candidate for the clinical management of MPE. However, intravenous administration of bevacizumab requires a much higher dose to achieve a corresponding effect, whose side-effect might offset the ability of bevacizumab to diminish effusion (18). Furthermore, although intrapleural administration is a standard approach for managing pleural effusion, intrapleural administration of bevacizumab has not yet been reported for the treatment of MPE as a consequence of NSCLC. In the present study, we evaluated the efficacy and safety of local intrapleural administration of a combination of bevacizumab with cisplatin for the treatment of MPE. The main purpose of this study was to seek a more efficient therapeutic approach for the clinical management of these cancer-associated diseases.

Materials and methods

Research study subjects. This study was approved by the institutional review board of the First Affiliated Hospital of Chinese PLA Postgraduate Medical School (Beijing, China) and written informed consent was obtained from all participants (Approval no. ML25391). Seventy-two NSCLC subjects (excluding squamous cell carcinoma) with a presence of excess levels of pleural fluid were enrolled in this study from August 2009 to December 2011 inclusive. The research study subjects included 44 males and 28 females with a mean age of 52.50 years (range, 66-82 years). Primary tumors included adenocarcinoma in 48 cases, alveolar cell carcinoma in 17 cases and large cell carcinoma in seven cases. The study subjects were randomized into the bevacizumab group (intrapleural therapy of bevacizumab combined with cisplatin; n=36), and the cisplatin group (intrapleural cisplatin therapy alone; n=36). Two study subjects in the cisplatin group withdrew, including one study subject who was lost to follow-up after two cycles of chemotherapy, and one study subject who presented with dual cancer during treatment.

There was no statistically significant difference (p>0.05) between the two groups in terms of gender, age, clinical stage and pathological subtype (Table I). The MPE in the study subjects had been confirmed by imaging such as X-ray, CT scan and type-B ultrasonic scan in addition to cytological examination. The enrolled study subjects showed various symptoms including chest distress, cough and dyspnoea, at different levels.

The inclusion criteria included the following: a) advanced NSCLC confirmed histologically or pathologically, but not including squamous cancer; b) chest X-ray, ultrasonography or CT scan showing large areas of unilateral or bilateral plural effusion; c) malignant tumor cells found in the pleural fluid; d) no intrapleural injection of antineoplastic drugs or hardener within one month of recruitment to the study; e) Kamofsky score (KPS) >60, age >18 years at time of recruitment, and a

predicted survival time >3 months; f) no major organ system dysfunction, and a blood cell count, as well as heart, liver and kidney test results within a normal range; g) previous chemotherapy to have been discontinued for >6 weeks prior to admission to this study.

The exclusion criteria included: a) history of allergy to biological agents; b) under current treatment with other antineoplastic agents; c) no detectable lesions; d) uncontrolled metastasis to the central nervous system; e) major organ dysfunction, such as congestive heart failure, malignant arrhythmia, angina requiring long-term medication, heart valve diseases, myocardial infarction and refractory hypertension; f) pregnant or breastfeeding women; g) infected wound; and h) history of refractory psychiatric diseases.

The criteria for withdrawal included: a) patients requesting withdrawal; b) stage III/IV adverse reactions related to bevacizumab therapy; c) poor study subject compliance; and d) disease progression. In addition, bevacizumab was discontinued if any of the following conditions occurred: a) wound dehiscence that needed intervention and associated complications; b) severe bleeding; c) severe arterial thrombus; d) hypertension crisis or hypertensive encephalopathy; e) reversible posterior leukoencephalopathy syndrome; and f) nephrotic syndrome.

Treatment protocol. After draining the pleural fluid by thoracentesis, study subjects were given intrapleural administration of either a combination of 30 mg of cisplatin plus 300 mg of bevacizumab or 30 mg of cisplatin monotherapy. After 2 h of bed rest, study subjects were asked to turn over every 15 min, in order to encourage full access of the delivered drugs to the chest wall. This treatment was given every two weeks. The tumor markers were examined at the end of the first treatment cycle. Detailed records of the responses following treatment were made by weekly blood monitoring and type-B ultrasonic tests. The end point of this study was the completion of 3 cycles of treatment. One week after the first intrapleural therapy, the enrolled NSCLC study subjects were given more than 3 cycles of conventional chemotherapy comprising paclitaxel (175 mg/m^2) and carboplatin (AUC=6).

Evaluation of the standard of efficacy and safety. Recent objective responses were determined according to a previous study (19). Complete remission (CR) was considered when the accumulated fluid had disappeared and was stable for at least four weeks; partial remission (PR) was considered when >50% of the accumulated fluid had disappeared, symptoms had improved, and the remaining fluid had failed to increase for at least four weeks; remission not obvious (NC) was considered when <50% of the accumulated fluid had disappeared; progression (PD) was considered when the accumulated fluid had increased. The total efficiency was calculated by taking the sum of CR+PR. At the same time, the median progression-free survival (PFS) and the median overall survival (OS) were assessed. Adverse reactions were evaluated by the Common Toxicity Evaluation Criteria (CTC) according to the National Cancer Institute (NCI). Qualify of Life (QOL) was evaluated by the Kanofsky score (KPS).

Quantitation of VEGF by enzyme-linked immunosorbent assay (ELISA). Pleural fluid was centrifuged at 4,000 rpm for

Table I. Comparison of the clinical characteristics of the evaluable NSCLC study subjects of the two treatment groups.

	Bevacizumab (%) n=36	Cisplatin (%) n=34	P-value
Age			
≥50	20 (55.56)	18 (52.9)	>0.05
18-50	16 (44.44)	16 (46.7)	>0.05
Gender			
Male	19 (52.8)	19 (55.6)	>0.05
Female	17 (47.2)	15 (444.1)	>0.05
Clinical stage			
IV-M1a[a]	25 (69.4)	24 (70.6)	>0.05
IV-M1b	11 (30.6)	10 (29.4)	>0.05
Smoker			
Ever smoker	22 (61.1)	21 (61.8)	>0.05
Never smoker	14 (38.2)	13 (38.2)	>0.05
EGFR mutation			
Wild-type	21 (58.3)	18 (52.9)	>0.05
Mutand	15 (41.7)	16 (47.1)	>0.05
Subtype			
Adenocarcinoma	23 (63.9)	22 (64.7)	>0.05
Alveolar cell carcinoma	9 (25.0)	9 (26.5)	>0.05
Large cell carcinoma	4 (11.1)	3 (8.8)	>0.05
Kamofsky score			
≥80	27 (75.0)	25 (73.5)	>0.05
>60 - <80[a]	9 (25.0)	9 (26.5)	>0.05
Systematic treatment history (other regimen)			
Yes	25 (69.4)	23 (67.6)	>0.05
No	11 (30.6)	11 (32.4)	>0.05
Local infusion history			
Bleomycin monotherapy	25 (69.4)	23 (67.6)	>0.05
No	11 (30.6)	11 (32.4)	>0.05

[a]Karnofsky scores were decreased by large amounts of pleural fluid.

10 min at 4°C, following which the supernatant was collected and assessed by ELISA using the VEGF-A ELISA kit (USCN), according to the manufacturer's instructions. The assay plates were read using a Microplate Reader (Bio-Rad, model 550). Carcinoembryonic antigen (CEA) was determined by a chemiluminescence method.

Quantitative RT-PCR for VEGF-A. Cells (~10^5-10^6) were subjected to total RNA extraction using TRIzol reagent (Invitrogen Corp., Carlsbad, CA, USA). For RT-PCR, first-strand cDNA was synthesized from 0.2 to 1.0 μg of total RNA with an oligo-dT primer (Gibco), according to the manufacturer's instructions. The sequences of the VEGF-A primers were: forward 5'-ctacctccaccatgccaagt-3', reverse 5'-aaatgctttc tccgctctga-3'.

Statistical analysis. Results are expressed as the means ± standard error of the mean. Data were analyzed by the χ^2 test and the rank sum test using SPSS 11.0 software. p<0.05 was considered to indicate a statistically significant difference.

Table II. Comparison of the efficacy between the bevacizumab and cisplatin groups.

Group	n	CR	PR	NC/PD	OR (%)
Cisplatin	34	2	15	17	50.00
Bevacizumab	36	17	13	6	83.33

CR, complete remission; PR, partial remission; NC/PD, remission not obvious/progression; OR, overall response.

Results

Short-term effect. The 70 evaluable enrolled study subjects had completed at least one cycle of treatment. The response rate for the combination therapy group was 83.33% (30/36 study subjects), while that for the cisplatin alone therapy group was 50.00% (17/34 study subjects). There was a statistically significant difference between the two groups in terms of

Table III. Treatment cycles of intrapleural infusion in the bevacizumab and cisplatin groups.

Group	Treatment cycle	Response (n)	CR	PR	Percentage (%)
Bevacizumab (n=36)	2	13	5	8	36.11
	3	15	11	4	41.67
	≥4	2	1	1	5.56
Cisplatin (n=34)	2	2	0	2	5.88
	3	4	0	4	11.77
	≥4	11	2	9	32.35

CR, complete remission; PR, partial remission.
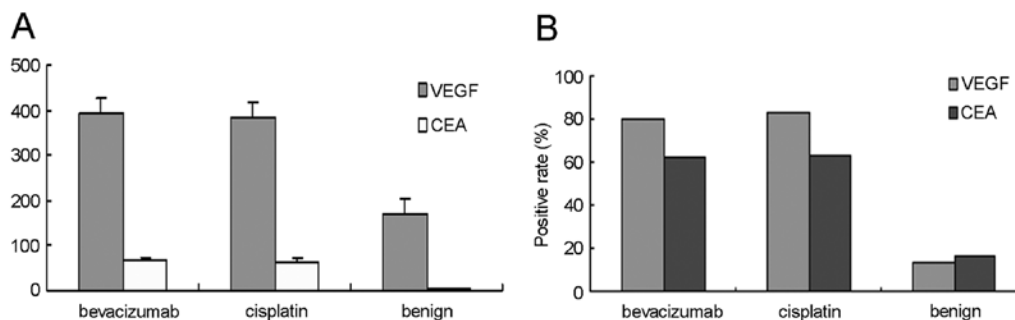
Figure 1. Comparison of progression-free survival (PFS) (A) and median overall survival (OS) (B) between the bevacizumab and cisplatin groups. (A) PFS: group 1, bevacizumab + cisplatin; group 2, cisplatin 5.3 vs. 4.5 months; p<0.05; (B) OS: group 1, bevacizumab + cisplatin; group 2, cisplatin 10.3 vs. 10.1 months; p>0.05. VEGF, vascular endothelial growth factor; CEA, carcinoembryonic antigen.

stratification of MPE (p<0.05) (Table II). Following 2-3 cycles of treatment, the response rate was significantly increased in the bevacizumab group (Table III). However, the response rate only increased after 4 or more cycles of treatment in the cisplatin monotherapy group (Table III). Statistical analysis was not performed here due to the relatively small case numbers.

Long-term effect. The median PFS was found to be 5.3 months for the bevacizumab combination group and 4.5 months for the cisplatin alone group. This observation indicated that the addition of bevacizumab to the therapeutic regime could significantly extend the PFS of the study subjects presenting with MPE (p<0.05) (Fig. 1A). The median survival time was found to be 10.3 and 10.1 months, respectively, in the two groups (p>0.05) (Fig. 1B).

Quality of life (QOL). Of the 36 study subjects in the combination therapy group, 30 study subjects were found to have an improved QOL (83.33%), one study subject showed a stable QOL, and one study subject showed a reduced QOL. In the cisplatin monotherapy group, only 50.00% (15 cases) of the study subjects showed an improvement in their QOL.

Toxicity. There was no evidence of significantly increased toxicity following systemic chemotherapy in combination with intrapleural therapy. The major side-effects of bevacizumab treatment included bleeding and high blood pressure. Although the study subjects in both groups presented symptoms such as neutropenia, nausea, vomiting and diarrhea, all of the symptoms were typical side-effects of chemotherapy rather than being exclusively associated with bevacizumab treatment. Although one of the study subjects showed nasal mucous membrane bleeding, this observation did not affect follow-up treatment. The incidence of hypertension was also significantly higher in the combination therapy group (p<0.05). However, observation of hypertension in this group fell into stage I and II (although one case required oral antihypertensive therapy) and no subject withdrew from the study due to toxicity. No significant difference in grade III-IV adverse side-effects was seen between the two groups (Table IV).

Levels and positive rate of VEGF-A and CEA. The levels and positive rates of VEGF-A and CEA seen in the pleural effusion are shown in Fig. 2. The difference between the two groups of NSCLC study subjects treated with either cisplatin monotherapy or combined therapy with bevacizumab and cisplatin was not statistically significant (p>0.05). The tumor marker CEA showed a significant difference between the study subjects presenting with MPE and those presenting with benign effusion (p<0.01). Treatment with bevacizumab and cisplatin significantly decreased VEGF levels in the pleural fluid compared to that of the cisplatin monotherapy group before and after the respective treatments (p<0.01).

Relationship between VEGF expression and response rate. The VEGF positive status of the study subjects was deter-

Table IV. Comparison of toxicity between the bevacizumab and cisplatin groups.

| Group	Toxicity	Grade				
		0	1	2	3	4
Bevacizumab (n=36)	Leucocytopenia	12	9	10	4	1
	Nausea and vomiting	19	12	5	0	0
	Diarrhea	30	4	2	0	0
	Rhinorrhagia	34	2	0	0	0
	Hemoptysis or gastrointestinal bleeding	36	0	0	0	0
	Hypertension	27	7	2	0	0
	Proteinuria	34	2	0	0	0
Cisplatin (n=34)	Leucocytopenia	12	8	9	4	1
	Nausea and vomiting	17	12	4	1	0
	Diarrhea	28	3	2	1	0
	Rhinorrhagia	34	0	0	0	0
	Hemoptysis or gastrointestinal bleeding	34	0	0	0	0
	Hypertension	32	2	0	0	0
	Proteinuria	34	0	0	0	0
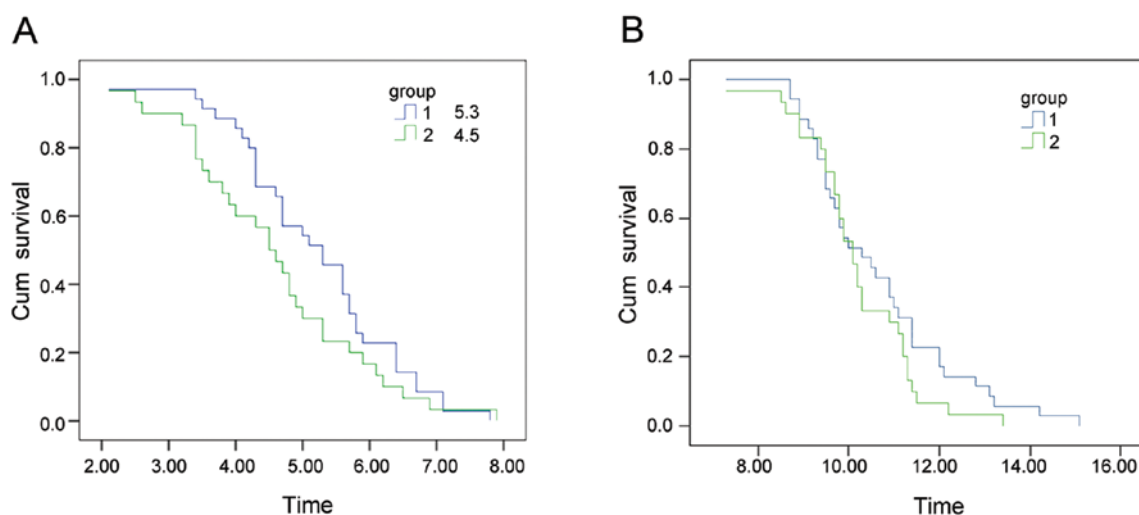
Figure 2. Levels of vascular endothelial growth factor (VEGF) (A) and carcinoembryonic antigen (CEA) (B) in the pleural fluid of NSCLC study subjects and benign pleural effusion subjects. The levels and positive rates of VEGF-A and CEA are shown. The difference between the two groups of NSCLC study subjects treated with either cisplatin alone or combined bevacizumab with cisplatin was not statistically significant (p>0.05). The tumor marker CEA showed a significant difference between the study subjects presenting with malignant pleural effusion and the subjects presenting with benign effusion (p<0.01).

mined when the pleural VEGF was higher than the normal maximum value of 300.6 pg/l. This was a value generated from a previous study which included 30 patients presenting with benign pleural effusions (unpublished data). The CEA-positive status was determined by comparison with the conventional standard value of pleural effusion (5 ng/l).

Bevacizumab was particularly efficient in the treatment of study subjects that were VEGF-positive (p<0.01) (Table V). Of the six VEGF negative study subjects, five showed no response to bevacizumab treatment, which suggested that bevacizumab therapy was specific to VEGF-positive patients. There were also five study subjects who were negative for CEA, but positive for VEGF yet showed an acceptable response to bevacizumab therapy (Table V). Of the 15 CEA-negative study

Table V. Relationship between efficacy and expression of VEGF and CEA in the bevacizumab group.

	VEGF (n=36)		CEA (n=36)	
	Positive	Negative	Positive	Negative
CR	17	0	12	5
PR	12	1	8	5
NC/PD	1	5	1	5

VEGF, vascular endothelial growth factor; CEA, carcinoembryonic antigen. CR, complete remission; PR, partial remission; NC/PD, remission not obvious/progression.
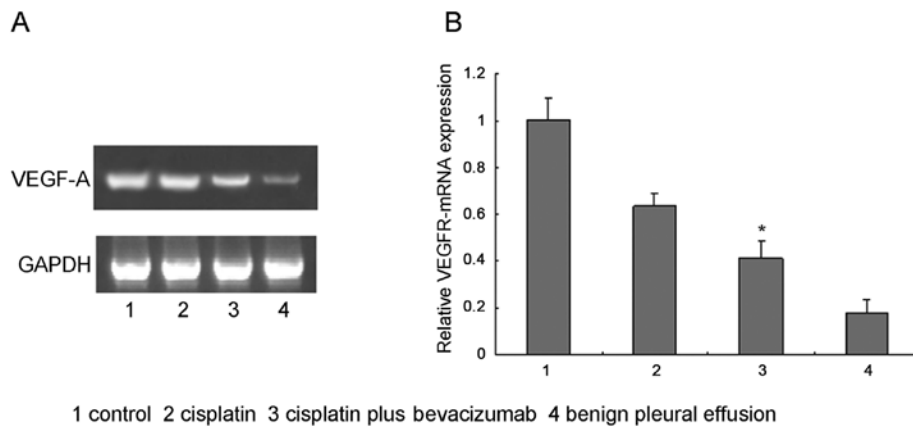
Figure 3. VEGF-A mRNA expression levels in the pleural fluid of the various groups. (A and B) The mRNA expression of VEGF-A in the pleural effusion is shown. Compared to study subjects treated with cisplatin monotherapy, the pleural VEGF-A mRNA level was significantly lower than in study subjects treated with combination therapy ($p<0.05$) and as determined by real-time RT-PCR.

subjects, five were refractory to bevacizumab, while among the bevacizumab-refractory study subjects, five presented as CEA-positive, indicating that CEA was not a specific marker for bevacizumab treatment. Analysis of the 25 study subjects who were initially resistant to intrapleural therapy with bleo-mycin revealed that combination therapy with bevacizumab plus cisplatin provoked highly responsive regression of pleural effusion in 22 study subjects, an observation which may support an association between the VEGF-positive status and the actual response of study subjects to bevacizumab.

mRNA expression of VEGF-A in pleural effusion. The pleural VEGF-A mRNA was significantly lower in study subjects treated with combination therapy as compared with subjects treated with cisplatin alone, as determined by real-time RT-PCR (Fig. 3). The expression levels of VEGF-A mRNA (log copy number/μg) normalized to the internal control GAPDH in samples without therapy (before therapy), with cisplatin monotherapy and with combined cisplatin plus bevacizumab therapy were found to be 0.54 ± 0.12, 0.24 ± 0.041 and 0.15 ± 0.029, respectively. Expression of VEGF-A was significantly lower following treatment with either cisplatin monotherapy or with combined cisplatin plus bevacizumab ($p<0.05$) (Fig. 4). There was also no difference in the basal expression of VEGF-A in both treatment groups ($p=0.97$) (Fig. 4). In addition, both treatment approaches were found to markedly decrease the mRNA expression levels of VEGF-A ($p<0.01$) in the study subjects. However, the extent to which VEGF-A mRNA decreased was much greater in subjects that had received the combination therapy compared to the subjects that had received cisplatin monotherapy ($p<0.01$). This observation suggested that bevacizumab could significantly reduce the levels of VEGF-A mRNA.

Discussion

Approximately 70-80% of patients with NSCLC are diagnosed at relatively advanced stages (stage IIIB to IV) of their disease, with a 5-year survival rate for NSCLC of less than 5% (1). Malignant pleural effusion (MPE) is seen in more than 50% of patients presenting with NSCLC (1,2). The mortality rate of
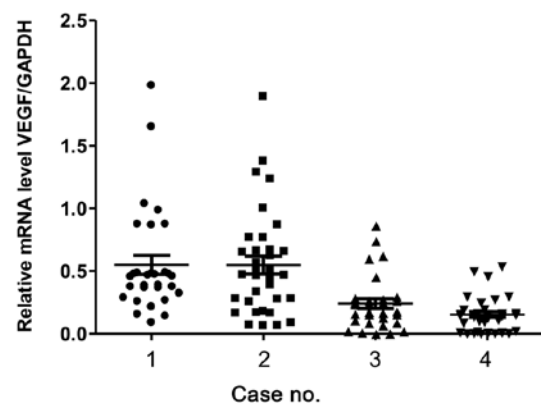

Figure 4. Ratiometric analysis of the VEGF-A mRNA/GAPDH levels in the pleural fluid of the various groups. VEGF-A expression was significantly lower following both treatments ($p<0.05$). Case 1, cisplatin group before treatment; 2, bevacizumab group before treatment; 3, cisplatin group after treatment; 4, bevacizumab group after treatment. The results show that the VEGF-A mRNA/GAPDH levels were similar between the two groups before treatment ($p=0.97$). The VEGF-A mRNA/GAPDH levels in the bevacizumab group were significantly decreased as compared to the levels prior to treatment and compared to those of the cisplatin-treated group ($p<0.01$).

patients with MPE is very high (19). The detection of MPE can be a clinical indicator of local invasion or systemic metastasis of advanced lung cancer.

The current mode of therapy for patients with MPE involves primarily local treatment, such as tube drainage, chemical pleurodesis and intrapleural administration of antineoplastic agents, accompanied by the addition of a diuretic and albumin, and restricted intake of salt and water. Among these therapeutic approaches, intrapleural infusion is an important approach for managing pleural effusion. The drugs that are used intrapleurally include cisplatin, which can directly target tumor cells and stimulate chemical pleurisy to reduce the pleural effusion (20). However, as the ability of intrapleural chemotherapy to penetrate the tumor is restricted to only a few millimetres (21), the response rates are often disappointing, with an effectiveness of 0-66% (22). Furthermore, the clinical outcome of current modes of therapy is unstable and while they can occasionally alleviate the symptoms, there is no eventual benefit in patient survival.

It has been demonstrated that in cancer patients the reabsorption of pleural fluid is blocked due to vascular and/or lymph node metastasis. By contrast, tumor cells can also secrete various growth factors which could lead to the local overexpression of VEGF, and thus contribute to elevated capillary permeability and fluid release from the capillary beds (10,23-27).

VEGF is a well-characterized angiogenic factor, with VEGF-A serving a prominent role in this regard, which on binding to its cognate receptors, primarily VEGFR-2, can increase vascular permeability, stimulate proliferation of vascular endothelial cells, promote the efflux of intravascular biological matrices, inhibit apoptosis of endothelial cells and activate enzymes that degrade the extracellular matrix. VEGF is thus able to facilitate the growth and extension of neovascularization. The ability of VEGF to promote vascular permeability renders it a key instigator of MPE (28,29). The elevated efflux of plasma proteins provides the microenvironment a suitable matrix for the formation of pleural effusions (30-32).

Bevacizumab is a humanized monoclonal antibody against VEGF-A, which is able to specifically block the binding of VEGF-A to its receptor, and, by doing so, induces the degradation of existing tumor blood vessels and normalization of the remaining blood vessels (33). Bevacizumab can also substantially suppress the continuous growth and metastasis of tumor cells by repressing angiogenesis in the tumor tissues (34). Bevacizumab has been approved and recommended by the FDA in the treatment of certain types of metastatic cancer, including colon, breast and kidney tumors, as well as glioblastoma multiforme. The role of bevacizumab in suppressing malignant progression of pleural effusion has only recently been elucidated, and suggests that treatment with bevacizumab, either as monotherapy or in combination with other chemotherapeutic agents, could be useful in the treatment of malignant pleural effusion (10,11). In addition, preclinical studies and clinical research, although still quite limited, have demonstrated that by inhibiting VEGF, treatment with bevacizumab may represent an effective way to prevent local fluid accumulation. To date, it has not been shown whether intrapleural bevacizumab therapy can be applied to MPE as a consequence of NSCLC.

On the basis of the aforementioned discussion, we compared the efficacy and safety of intrapleural combination therapy with bevacizumab and cisplatin with that of cisplatin monotherapy on MPE. Our results indicated that the intrapleural combination therapy of bevacizumab and cisplatin directed a very promising short-term effect, with a response rate of 85.71%, a value which was significantly higher than that of cisplatin monotherapy (56.67%).

Our results are consistent with a recent report that showed that pleural effusion could be markedly reduced by treatment with bevacizumab in combination with carboplatin and paclitaxel in a 63-year-old patient presenting with lung cancer (35). Similarly, intravenous or intraperitoneal administration of bevacizumab has been shown to be highly effective in the treatment of malignant ascites, at least in studies where only a limited number of study subjects were assessed (18,36-38).

We also showed that 88.57% of subjects presenting with both NSCLC and MPE overexpressed VEGF following treatment with bevacizumab. Additionally, study subjects that displayed originally high VEGF expression levels showed a more improved response than those subjects that were VEGF-negative.

The response rate for CEA-positive patients was found to be 57.1%, where five subjects that were negative for CEA but positive for VEGF showed a satisfactory response to bevacizumab. By contrast, among the bevacizumab-resistant subjects, three were found to be CEA-positive, and this observation indicated that CEA was not a specific marker for bevacizumab treatment.

Analysis of the 25 subjects who were initially resistant to chemotherapy showed that treatment with a combination of bevacizumab plus cisplatin provoked regression of pleural effusion in 22 of the 25 subjects. This observation further supported the hypothesis that bevacizumab is highly effective in the subjects that were considered VEGF-positive. Furthermore, in most cases, the pleural effusion was well controlled after one or two treatment cycles, and the stratification of effusion was reduced following three cycles of treatment.

The median progression-free survival (PFS) for both treatment groups showed a significant difference, and indicated that the addition of bevacizumab to the therapeutic regimen may significantly extend the PFS of patients with MPE. Although the patients receiving bevacizumab treatment survived longer, the median survival (OS) for the two groups did not show any statistically significant difference. This could be due to the difference of the second-line treatment that some of the study subjects received.

Quantitative analysis of VEGF mRNA suggested that there was no difference in basal VEGF expression between subjects that had received any therapy. However, there was a significant difference between the subjects that had received combination therapy and the subjects that were treated with cisplatin monotherapy.

We also found that the VEGF mRNA levels in patients administered bevacizumab treatment were significantly lower than those found in subjects treated with cisplatin monotherapy. This observation suggested that bevacizumab may have downregulated VEGF-A gene expression, thereby inhibiting cell proliferation and migration, factors that are required for neoangiogenesis. This finding also suggested that bevacizumab may have suppressed the permeability of blood vessels, thereby eliminating the accumulation of pleural fluid. We suggest that this is likely the underlying mechanism by which bevacizumab inhibits the formation of MPE. Our results further confirmed the clinical significance of detecting VEGF mRNA and protein as biomarkers for the clinical management of MPE with bevacizumab.

Overexpression of VEGF in tumor tissues is associated with the metastatic potential of tumors and their rate of survival (39,40). Thus, elevation in the serum levels of VEGF and overexpression of VEGF within the tumor tissue are considered to be associated with NSCLC staging and prognosis (41,42). Of note, MPE that is caused by NSCLC also shows substantial increases in the levels of VEGF and its concentration exceeds that found in serum. By contrast, pleural effusion that is caused by lung infections, heart failure or cirrhosis of the liver does not appear to upregulate VEGF (43-48). Additionally, previous studies have shown that VEGF is the most highly overexpressed cytokine among 21 cytokines and chemokines that have been identified in both benign and malignant pleural effusion (31,49,50). Markedly,

the concentration of VEGF found in MPE can be considered an independent prognostic factor for tumor PFS and total survival. VEGF has been found to be associated with the sensitivity of tumors to chemotherapeutic agents. Therefore, treatment with an antibody specific to VEGF in patients with high levels of VEGF in the pleural effusion suggests that VEGF can be considered a biomarker for bevacizumab treatment of MPE (18,51,52).

Our study provides evidence that combination therapy with intrapleural bevacizumab and cisplatin can efficiently treat MPE caused by NSCLC. This combination therapy can readily diminish effusion, with a positive health outcome for the patient. During this study, we did not detect any severe side-effects, indicating that this line of therapy is well tolerated. The combination therapy used in our study was able to control pleural effusion within a short period of time. Although mild hypertension was detected, it never exceeded grade II. We did not observe any proteinuria, thrombosis, gastrointestinal or pulmonary bleeding. Also, we did not have a single research study subject withdraw from the study due to toxicity. Although current evidence demonstrates that intrapleural bevacizumab does not lead to specific risks, we still recommend that some precautions be taken when managing patients with a significant tumor burden or in patients where squamous cell carcinoma is found in the main bronchus or in patients receiving surgery in the latter stages of the disease.

Treatment with intrapleural delivery of bevacizumab and cisplatin combination therapy is reliable, safe and feasible; it provides a novel approach for the management of MPE, and warrants further investigation. Our study demonstrated that the VEGF levels found in the pleural effusions of benign disease were markedly lower than those found in lung cancer. Moreover, VEGF expression can be significantly downregulated by intrapleural delivery of bevacizumab in lung cancer patients. This latter observation supports the theory that bevacizumab suppresses MPE by mechanisms dependent, in part, on the inhibition of VEGF expression.

We conclude that intrapleural combinatorial therapy with bevacizumab and cisplatin represents a novel treatment option for MPE. Intrapleural infusion does not necessarily impart an additional burden to the patients since it is also a routine therapeutic approach for pleural effusion. The preclinical and clinical study for intrapleural bevacizumab therapy has been successful and has also yielded some promising outcomes (53,54). Our findings provide further evidence to an accumulating body of studies supporting the use of bevacizumab therapy, and suggest that intrapleural bevacizumab therapy could provide long-lasting effects in patients with MPE associated with advanced NSCLC. In addition, our investigation suggests that biomonitoring VEGF expression levels could be a reliable bioindicator of the clinical effectiveness of bevacizumab therapy.

Acknowledgements

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