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Original Article

Endostatin improve the effect of radiotherapy in human NSCLC patients with brain metastases

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Abstract: Brain metastasis in non-small cell lung cancer (NSCLC) patients is the main reason of the failure of the treatment of NSCLC. Combination of anti-angiogenesis agents and radiotherapy is a newly way in therapy of NSCLC. In the present study we investigated the clinical efficacy of recombinant human endostatin combined with radiotherapy on human NSCLC patients with brain metastases. NSCLC patients with brain metastasis were treated with radiotherapy or radiotherapy combine with endostatin, the short-term efficacy, overall survival time, cerebral edema index and adverse reactions were observed. The expression level of vascular endothelial growth factor receptor2 (VEGFR 2) in primary lesions was detected by using immunohistochemical analysis. The results indicated that the symptom of brain edema was alleviated and no severe adverse reactions were yielded in the combination group compared with single radiotherapy group. The short-term clinical efficacy was enhanced in total population, whereas no statistical significance was noted. However, there was statistical significance in the patients with positive VEGFR 2 in terms of short-term clinical efficacy. Regarding overall survival time, there was no statistical significance in the total population or in the patients with positive VEGFR 2. No clinical benefits were obtained in terms of median survival time. Taken it together our studies demonstrated that compared with radiotherapy alone, recombinant human endostatin combined with radiotherapy can relieve brain edema in the patients with lung cancer with brain metastasis and obtain better short-term clinical efficacy in the population with positive VEGFR 2.

Keywords: Endostatin, radiotherapy, VEGFR 2, NSCLC, brain metastases

Introduction

Brain is the most frequent organ of metastasis in non-small cell lung cancer (NSCLC) patients; this is the main reason of the failure of the treatment of non-small cell lung cancer. Nowadays whole brain radiotherapy (WBRT) remains the most common approach for the treatment of brain metastasis [1]. RTOG9805 findings revealed that precise radiotherapy plus WBRT could enhance the survival of patients with simple metastasis; however the overall prognosis remains to be unsatisfactory [2]. Hypoxic tumor microenvironment and peritumoral edema are the primary causes of brain metastasis. Endostatin (ES), an anti-angiogenesis agent, can not only improve the hypoxic tumor microenvironment, but also alleviate peritumoral edema via inhibiting vascular endothelial growth factor (VEGF) and vascular endothelial growth factor receptor2 (VEGFR 2) pathways. However the combination of VEGFR2 inhibitor and radiotherapy is still poor studied.

In this study, patients with non-small cell lung cancer and brain metastasis were selected and ES administration in combination with radiotherapy was applied in the treatment of these patients. We try to investigate the benefits of ES and radiotherapy combination therapy.

Materials and methods

Study subjects

80 patients diagnosed with brain metastasis of NSCLC by using MRI imaging between January 2011 and January 2013 were enrolled in this clinical trial. Detailed clinical data and grouping method were illustrated in **Table 1**. Until March 2013, all patients completed the whole treat-

Table 1. Clinical data of 80 patients with lung cancer with brain metastasis

Category	Combined therapy group	Radiotherapy alone group	*P* value
Gender			>0.05
Male	21	22	
Female	19	18	
Age (year)			>0.05
Range	57-75	59-75	
Median	67	64	
Pathological type of primary cancer			>0.05
Adenocarcinoma	23	21	
Squamous cancer	17	19	
Number of brain metastasis			>0.05
Multiple	19	21	
Simplex	21	19	
Sites of brain metastasis			>0.05
Supratentorial	26	23	
Infratentorial	14	17	
Clinical manifestations			>0.05
Intracranial hypertension	17	18	
Nervous targeted signs	33	35	
Psychiatric symptoms	7	5	
Severity of brain edema			>0.05
Mild	11	11	
Moderate	15	13	
Severe	14	16	
Control of primary lesions			>0.05
CR+PR	22	20	
SD	11	12	
PD	7	8	
Median KPS	70	70	>0.05

ment. The range of follow-up time was 1-53 months (median follow-up: 12 months). No cases were loss to follow-up with a follow-up rate of 100%.

Inclusion, exclusion and withdrawal criteria

Inclusion criteria: Patients with brain metastasis of NSCLC upon the first treatment (only limited to brain metastasis regardless of the number of metastasis and whether the primary lesion was treated or not); patients with significant nerve and/or mental symptoms; aged ≤75 years; KPS grading >60; estimated survival ≥3 months; those patients who are willing to participate in this study. Informed consents were obtained from all participants. This study has been approved by the ethnic committee of our hospital. Flow chart for patients recruitment was illustrated in **Figure 1**.

Exclusion criteria: Patients presenting with other sites of metastasis; those complicated with severe heart disease and abnormal electrocardiogram detection.

Withdrawal criteria: During the treatment, the patients presented with intolerable toxicity and serious hemorrhage reaction.

Study design

It is a random control and phase II clinical trial. A stratified random control method was utilized to assign all patients into the combined therapy and radiotherapy alone groups. Local control rate of NSCLC patients with brain metastasis was regarded as the end-point event. A total of 80 patients were included.

Treatment method

Radiotherapy: The radiotherapy procedures were the same between two groups. Whole brain radiotherapy technique was adopted (Siemens Healthcare, Forchheim, Germany) using 6MV X-ray linear accelerator, 3Gy/time, 5 times/week for a total of 10 times. For a single lesion, topical 3D-CRT or IMRT was performed with a PTV dose of DTI0 Gy/5 times, 2 Gy/time, 5 times a week. The radiotherapy plan and evaluation of critical organ were mainly optimized by DVH images.

Administration of endostatin: The endostatin (Simcere, China) was transfused by infusion pump with a dosage of 7.5 mg/m/d, simultaneous to the delivery of radiotherapy.

Immunohistochemical test of VEGFR 2

The samples were collected from primary lung lesion surgery or puncture specimen for subse-

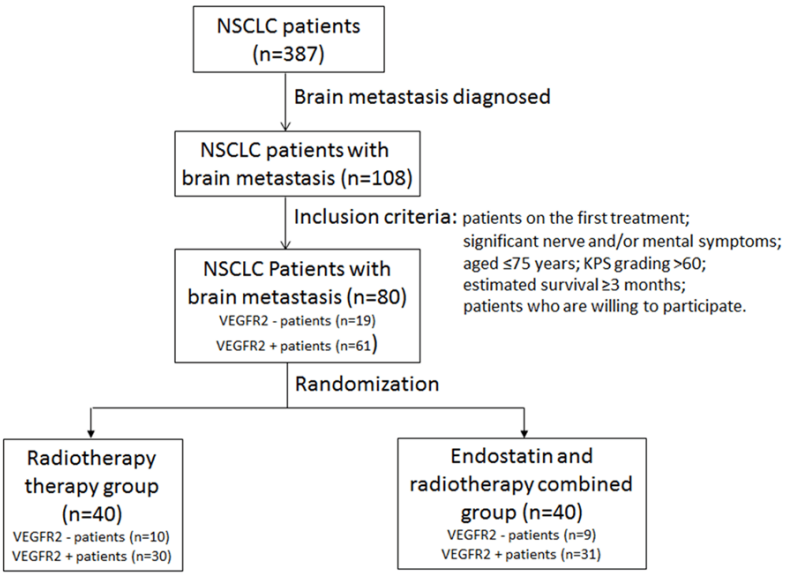

Figure 1. Flow chart for patients recruitment.

quent use of immunohistochemical detection. Evaluation criteria: the percentage of positive cells ≤10% was deemed as grade 0, 10%-50% as grade I, 51%-79% as grade II and ≥80% as grade III; the staining coloring: no staining was regarded as grade 0; weak positive as grade I, slight yellow; positive staining as grade II, yellow; strong positive as grade III, dark-brown [3]. Staining index counting method was employed. Staining index = classification of positive cell percentage × classification of staining intensity. Staining index ≥3 points was deemed as positive immune reaction.

Observation index

Short-term efficacy: Based upon the Response Evaluation Criteria in Tumors 1.0, the assessment criteria of short-term efficacy can be divided into complete response (CR), partial response (PR), stable disease (SD) and progress disease (PD). The efficacy rate equals to the sum of CR and PR. The clinical efficacy between two groups was evaluated at 4 weeks after respective treatment.

Overall survival (OS): The survival starting from the time when random grouping to the final follow-up or death. The loss to follow-up was treated by truncated method.

Brain edema index: On the basis of brain MRI, the edema index (EI) was calculated from the formula: EI= volume of edema and tumor/volume of tumor. EI=1 denoted no peritumoral edema, 1<EI≤ 1.5 as slight degree of edema, 1.5<EI≤2 as moderate degree of edema and EI>2 as severe degree of edema [4].

Adverse reactions: The evaluation criteria of acute radiation-induced injury by Radiation Therapy Oncology Group (RTOG) were adopted in this study.

Grading of quality of life was assessed by EORTC QLQ-LC43 [5, 6].

Statistical analysis

SPSS 17.0 statistical software was employed for data analysis. Enumeration data and group comparison were conducted by chi-square test. Measurement data were expressed as means ± SD. The mean values among groups were statistically compared by using *t*-test. P<0.05 was considered as a level of statistical significance.

Results

Immunohistochemical analysis of primary VEGFR2

VEGFR2 was mainly expressed in tumor cells and the cytoplasm and cell membrane of blood vessel endothelial cells, which were stained as yellow-brownish or dark brown color (**Figure 2**), no staining was observed in the normal lung tissue. Immunohistochemical analysis revealed that the positive rate of VEGFR2expression in lung cancer tissues was 76.2% (61/80). The positive rate in the combined therapy group was 77.5% (31/40) and 75% in the radiotherapy alone group (30/40).The positive rate for males was 79% and 73% for female counterparts with no significant difference (P=0.52). The positive rate for squamous cancer patients achieved up to 80% and 72% for adenocarcinoma subjects (P=0.44). Patients with simple brain metastasis had a positive rate of 72% and 80% for those with multiple metastasis (P=0.43). The positive rate in patients with supratentorial

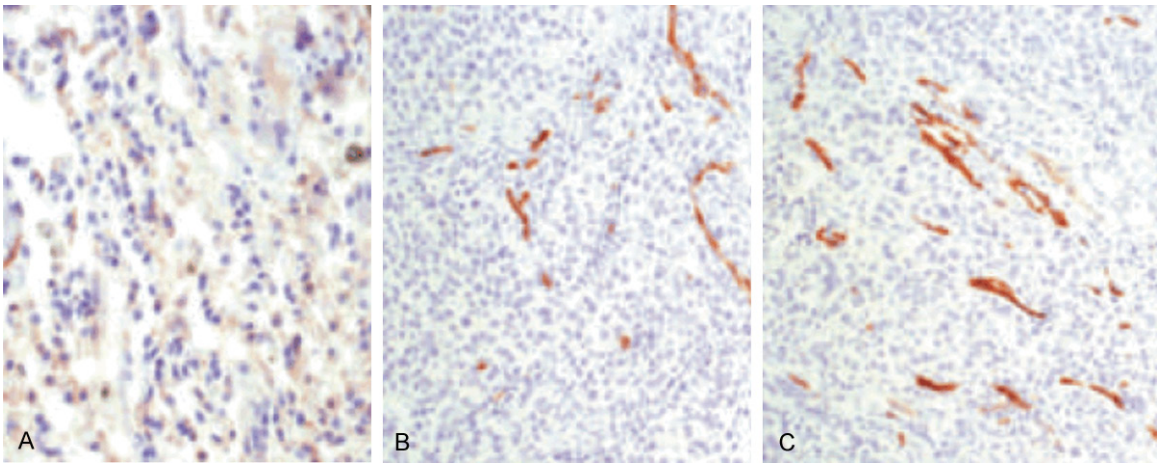

Figure 2. The expression of VEGFR2 in human lung cancer by Immunohistochemical staining assay. A: Negative VEGFR2 expression in lung cancer tissue. B, C: Positive VEGFR2 expression in lung cancer tissue.

Table 2. Comparison of cerebral edema index between groups in 80 patients

Treatment group	Cerebral edema index	*P* value
a Radiotherapy group before treatment	2.37 ± 1.14	
b Radiotherapy group after treatment	2.02 ± 0.98	b vs d: P<0.05, t=4.90
c Combined therapy group before treatment	2.39 ± 1.25	
d Combined therapy group after treatment	1.22 ± 0.34	d vs c: P<0.05, t=5.67

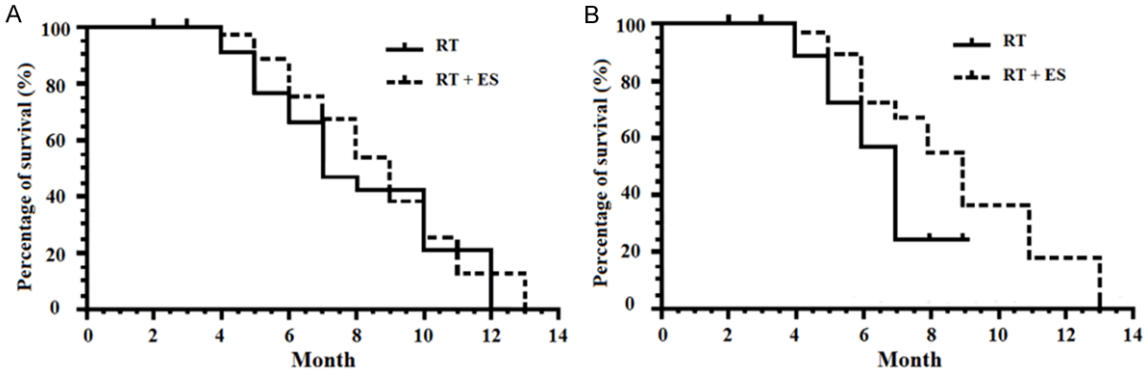

Figure 3. The survival curve of patients in each therapy group. A. Total population (n=80), survival curve between two groups (OS, P=0.35, HP=0.777, 95% CI: 0.25-1.30). B. Among 61 patients with positive expression of VEGFR2, survival curve between two groups (OS, P=0.109, HP= 0.875, 95% CI: 0.40-1.34).

metastasis was 76% and 77% for those with infratentorial metastasis (P=0.84). The positive rate in patients with moderate or severe degree brain edema was 89%, significantly higher compared with 41% in those with slight degree of brain edema (P=0.000). The positive rate for patients with SD+PD was up to 81%, significantly higher than 52% in their counterparts with CR+PR (P=0.003).

Comparison of short-term clinical efficacy between two groups

Among 80 patients, the overall efficacy (CR+PR) in the combined therapy group was 90% and 75% in the radiotherapy alone group with no statistical significance (χ^2=3.11, P=0.07). Among the 61 patients with positive VEGFR2, the overall efficacy in the combined group was

Table 3. Comparison of cerebral edema index betweengroups in 61 patients with positive expression of VEGFR2

Treatment group	Cerebral edema index	*P* value
a Radiotherapy group before treatment	2.34 ± 1.08	
b Radiotherapy group after treatment	2.06 ± 0.98	b vs d: P<0.05, t=4.17
c Combined therapy group before treatment	2.70 ± 1.25	
d Combined therapy group after treatment	1.22 ± 0.34	d vs c: P<0.05, t=5.80

Table 4. Comparison of toxic reaction between the combined therapy and radiotherapy alone groups

Adverse reactions	Combined therapy group (n)				Radiotherapy alone group (n)				*P* value
	0	I	II	III	0	I	II	III	
Arrhythmia	32	8	0	0	40	0	0	0	>0.05
Heart function	40	0	0	0	40	0	0	0	>0.05
Risk of bleeding	40	0	0	0	40	0	0	0	>0.05
Neutropenia	30	8	2	0	36	2	2	0	>0.05
Thrombocytopenia	36	2	2	0	38	2	0	0	>0.05
Anemia	40	0	0	0	40	0	0	0	>0.05

Note: I, II, III denotes the classification criteria of common toxic responses of NCIC-CTG agents.

93% and 67.7% in the radiotherapy alone group with a statistical significance (χ^2=6.31, P=0.012).

Comparison of EI and OS before and after treatment between two groups

Among 80 patients, the EI before treatment in the combined group was 2.39 ± 1.25 and 2.37 ± 1.14 in the radiotherapy alone group (*t*=1.44, P>0.05); the EI after therapy in the combined therapy group was 1.22 ± 0.34 and 2.02 ± 0.98 in the radiotherapy alone group (*t*=5.67, P<0.05) (**Table 2**). The median survival in the combined therapy group was 9 months and 7 months in the radiotherapy alone group (P=0.35, HP=0.777, 95% CI: 0.25-1.30) (**Figure 3A**). Among 61 patients with positive expression of VEGFR2, the EI before treatment in the combined group was 2.7 ± 1.25 and 2.34 ± 1.08 in the radiotherapy alone group (*t*=1.07, P>0.05); the EI after therapy in the combined therapy group was 1.22 ± 0.34 and 2.06 ± 0.98 in the radiotherapy alone group (*t*=5.80, P<0.05) (**Table 3**). The median survival in the combined therapy group was 8 months and 7 months in the radiotherapy alone group (P=0.109, HP=0.875, 95% CI: 0.40-1.34) (**Figure 3B**).

Quality of life and toxic reaction

The patients in the combined therapy group were not affected. The incidence of toxic events did not significantly different between two groups (P>0.05), as illustrated in **Table 4**.

Discussion

The combined therapy of anti-angiogenesis agents and radiotherapy is a newly proposed concept in recent years. It has been traditionally suggested that the use of anti-angiogenesis drugs may worsen the hypoxic severity in tumor tissues, which suppresses the efficacy of radiotherapy. However, multiple studies have demonstrated that administration of anti-angiogenesis agents can increase the efficacy of radiotherapy upon various types of malignant tumors, normalize the vascular system of tumors, prevent the effusion of hemostasis catheter, alleviate tumor tissue edema, ease tumor hypoxia, thereby enhancing the tumor sensitivity towards radiotherapy [7-9]. In this study, albeit the use of endostatin could increase the short-term efficacy in treatment of lung cancer with brain metastasis, but no statistical significance was observed. However, the patients with positive expression of VEGFR2 obtained benefits from administration of endostatin, suggesting that endostatin is probably beneficial to certain population. The synergistic mechanism is possibly associated with VEGFR2.

Endostatin is a small molecule protein with multiple target sites. It plays a role in the regulation of angiogenesis mainly via blocking the phosphorylation of VEGFR2 tyrosine kinases on endothelial cells [10]. Endostatin is mainly distributed within blood vessel endothelial cells. Recent studies revealed that VEGFR2 is equally expressed in malignant tumor cells, especially

NSCLC [11] and breast cancer cells [12], etc. In this study, immunohistochemical test detected that the positive rate of VEGFR2 expression in the lung cancer tissues was 76.2%, which is not only expressed in cytoplasm, but also on the cell membrane. The positive rate found in our study is significantly higher compared with the findings from previous studies [13], probably because the subjects enrolled in this study are in advanced stage. By stratified analysis, the positive rate is not correlated with patients' gender, pathologic type and number and sites of metastasis, which is consistent with previous reports [14]. The positive rate is associated with the severity of brain edema and the control of primary tumors instead, hinting that positive expression of VEGFR2 might be correlated with the malignancy of tumors.

Is the VEGFR2 expression correlated with brain edema? Recent studies found that brain metastasis complicated with brain edema is mainly associated with VEGFR2 besides VEGF [15]. VEGFR2 within the tumor cells bind with foreign VEGF. It not only reacts with its receptors, but also with the receptors located on the tumor blood vessel endothelial cells, leading to increased permeability of blood vessels, which is the first leading cause of edema of brain metastasis tumors [16]. In this study, EI was adopted to evaluate the clinical efficacy of brain edema. For patients with positive VEGFR2, the severity of brain edema was significantly alleviated after use of endostatin compared with their counterparts in the radiotherapy alone group. However, the median survival of patients with high level of VEGFR2 was only 8 months, lower compared with 9 months of the overall population, suggesting that patients with high expression of VEGFR2 have poor prognosis, which is a factor resistant to the radio- and chemo-therapy. These findings are consistent with previous evidence [11]. A variety of confounding factors affect OS, especially GPA classification. In this study, the sample size is relatively small, probably leading to statistical error. A larger sample size study is urgently required.

Taken together, endostatin in combination with radiotherapy is able to significantly enhance the clinical efficacy in treatment of lung cancer with brain metastasis for a specific population, obviously alleviate brain edema, enhance short-term efficacy, whereas yield no severe complications and toxic reaction. However, the long-term survival rate is not significantly enhanced, which remains to be further explored. The population with high expression of VEGFR2 protein within tumor cells may be suitable for the combined therapy of endostatin and radiotherapy.

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Disclosure of conflict of interest

None.

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