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Original Article

Efficacy, safety and tolerance of PEG-ENDO (an N-terminal mono-PEGylated recombinant human endostatin) with TC regimen (paclitaxel/carboplatin) therapy in the treatment of advanced non-small cell lung cancer: a single-center, open trial phase I study

Lie Ma*, Jiguang Meng*, Zhenqian Liu, Yongqun Li, Yan Zhang, Zhihai Han

*Department of Respiratory Disease, The Navy General Hospital of PLA, Beijing 100048, China. *Equal contributors.*

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Abstract: Purpose: The purpose of this study is to evaluate safety and tolerance of PEG-ENDO (an N-terminal mono-PEGylated recombinant human Endostatin) combined with TC (Paclitaxel/Carboplatin) in advanced NSCLC (non-small cell lung cancer) and to evaluate the efficacy of combinational anti-cancer therapeutics. Methods and patients: Between December 2012 and September 2014, 22 cases of advanced NSCLC patients were enrolled and treated by TC (first-line chemotherapy regimen) combined with PEG-ENDO of different dosages (7.5 mg/m^2, 10.0 mg/m^2, 12.5 mg/m^2, 15.0 mg/m^2). Safety and tolerance of PEG-ENDO with TC as well as the anti-cancer therapeutic efficacy were investigated. Results: 22 cases completed the programmed two cycles of therapy and were subjected to efficacy evaluation. The results showed that 5 cases were PR (partial response), 14 cases were SD (stable disease), 3 cases were PD (progressive disease), and the overall DCR (disease control rate) was 86.4%. There were 2 SAE (serious adverse event) cases in the highest 15.0 mg/m^2 PEG-ENDO treated group and no serious adverse events were reported in lower dosages of PEG-ENDO. Moreover, other adverse events were reversible. Conclusions: PEG-ENDO combined with TC chemotherapy has a certain effect for advanced NSCLC patients. The overall DCR of combination therapy is higher than that of chemotherapy alone. It is relatively safe and well-tolerated at lower doses, but there may be some risks at the higher dose (15.0 mg/m^2).

Keywords: PEG-ENDO, TC chemotherapy, NSCLC, efficacy, safety and tolerance

Introduction

In 1971, Dr. Folkman proposed the famous "tumor starvation" hypothesis that tumor growth is angiogenesis dependent [1]. Angiogenesis, the formation of new blood microvessels from pre-existing vessels, is a normal and vital process in growth, wound healing and development, as well as tumor growth and metastasis (Carmeliet and Jain 2011). Angiogenesis is central to the growth of cancer, especially when the size of tumor is beyond 1-2 mm^3, which can transport nutrient and oxygen for tumor cell proliferation and facilitate tumor cell invasion into circulation system and metastasize to distant organ [2, 3]. During tumor angiogenesis, quiescent endothelial cells become active under the stimulation of angiogenic factors such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), angiopoietin 1 and 2, interleukin-8, and platelet-derived growth factor-beta (PDGF-β) [4-6].

In normal tissues, the pro- and anti-angiogenic factors can form the balanced "angiogenesis switch" [7-9]. There are endogenous mammalian proteins that inhibit endothelial cell growth and may play a physiologic role in maintaining the normally low replication rate of vascular endothelial cells [10-14]. Inhibition of angiogenesis is a new valuable approach to cancer therapy. Endogenous specific inhibitors of angiogenesis include angiostatin, Endostatin, and

Table 1. Baseline demographics and disease characteristics (7.5 mg/m^2)

NO.	Retreatment	Sex	Age	PS	Smoking	Hypertension	Pathology	Stage	LN	Metastasis
A01	Y	Female	67	1	N	N	Squamous	IV	Y	Lung
A02	N	Female	46	1	N	N	Glandular	IV	Y	Pleura
A03	Y	Female	59	1	N	N	Glandular	IV	Y	Bone
A04	N	Female	66	1	Y	Y	Glandular	IV	N	Lung

PS: Performance status, LN: Lymph node metastasis.

Table 2. Baseline demographics and disease characteristics (10.0 mg/m^2)

NO.	Retreatment	Sex	Age	PS	Smoking	Hypertension	Pathology	Stage	LN	Metastasis
AA01	N	Male	66	1	N	N	Squamous/Glandular	IV	Y	Parenchyma
AA02	Y	Female	54	2	N	N	Glandular	IV	Y	Lung, Bone
AA03	N	Male	67	1	Y	Y	Squamous	IV	Y	Left lung
AA04	N	Male	53	1	Y	N	Squamous	IIIB	Y	N
AA05	N	Male	51	1	Y	N	Squamous	IIIB	Y	N
AA06	N	Female	66	1	N	N	Squamous/Glandular	IV	Y	Right lung
AA07	N	Female	68	1	Y	Y	Acinous carcinoma	IV	Y	Left lung

tumstatin [10]. They do not inhibit proliferation of resting confluent endothelial cells, nor tumor cells *in vitro.* Endostatin, a 20-kD fragment derived from the C-terminal region of collagen XVIII, was firstly isolated from the culture medium of a murine hemangioendothelioma cell line [15, 16]. The molecular mechanism studies of Endostatin anti-angiogenic function focused on its cell surface receptors. Endostatin can be internalized into the nucleus of active endothelial cells through its cell surface receptor nucleolin and integrins [17-19]. Treatment with recombinant Endostatin induced the regression of experimental tumors to dormant, microscopic lesions [15, 19], which strongly suggested that Endostatin is a promising anti-cancer drug candidate.

Although the clinical trials of wild type recombinant human endostatin was terminated in the United States, ZBP-Endostatin (zinc-binding peptide-Endostatin), a modified recombinant human Endostatin (rh-Endo) with an additional nine-amino acid sequence attached to the N-terminal of the wild type Endostatin, was approved by the State Food and Drug Ad-ministration of China (SFDA) in 2005 for the treatment of non-small-cell lung cancer [20]. In clinical setting, Phase I and II studies revealed that ZBP-Endostatin was effective as a single agent with good tolerance in pretreated advanced NSCLC patients at the dose of 7.5 mg/m^2 daily [21, 22]. Wild type endostatin used in the clinical trials of US was N-terminal truncated [23, 24]. Compared with the wild type, the additional nine-amino acid-sequence (MGGSHHHHH) was added at the N-terminal of the protein, which could be utilized to improve stability of the protein [24-26]. N-terminal integrity and correct protein refolding are two fundamental issues that influence the development of endostatin products.

The drug used in this study is PEGylated recombinant human Endostatin (code name: PEG-ENDO), which is a long-acting Endostatin. Many therapeutic proteins such as enzymes and cytokines have been significantly improved by PEGylation. It is known that covalent modification with PEG masks the surface of the protein and increases the molecular size of the polypeptide, thus decreasing immunogenicity and kidney filtration, and increasing solubility and *in vivo* residence time [27]. PEGylation also protects the protein from proteolytic enzymes, thus increasing its stability and prolonging its half-life *in vivo* [27, 28]. In tumor patients, the mean half-life of PEG-ENDO is 35.8 h in single dose (7.5 mg/m^2), 38.2 h (week 1) and 51.1 h (week 3) in multiple-dose (7.5 mg/m^2) (Jian Yang, et al., to be submitted). However, the half-life of ZBP-Endostatin is only 2.86 ± 0.53 h [29]. Thus, in contrast to the daily infusion of ZBP-Endostatin, PEG-ENDO is designed as weekly infusion. Therefore, we speculated that PEG-ENDO combined with TC chemotherapy may benefit the advanced NSCLC patients, which is one of the two major purposes

Table 3. Baseline demographics and disease characteristics (12.5 mg/m^2)

NO.	Retreatment	Sex	Age	PS	Smoking	Hypertension	Pathology	Stage	LN	Metastasis
AB01	N	Male	60	1	Y	N	LCLC	IV	Y	Lung
AB02	N	Male	59	1	Y	N	Squamous	IV	Y	Lung, Pleura
AB03	N	Male	56	1	Y	N	Squamous	IV	Y	Bone
AB04	N	Male	63	1	Y	N	Squamous	IV	N	Bone

Table 4. Baseline demographics and disease characteristics (15.0 mg/m^2)

NO.	Retreatment	Sex	Age	PS	Smoking	Hypertension	Pathology	Stage	LN	Metastasis
B01	N	Male	59	1	Y	N	Squamous	IIIA	Y	N
B02	N	Male	61	2	Y	Y	Squamous	IIIB	Y	N
B03	Y	Male	62	1	Y	N	Squamous	IV	N	Hydrothorax
B04	N	Male	41	1	Y	N	Squamous	IV	Y	Bone, Enterocoelia
B05	N	Male	69	1	Y	N	Squamous	IIIB	Y	N
B06	N	Female	39	1	N	N	Glandular	IV	Y	Pleura
B07	N	Male	53	1	Y	N	Squamous	III	Y	N
B08	N	Female	42	1	N	N	Glandular	IV	Y	Lung, Pericardium
B09	N	Male	62	1	Y	N	Glandular	IV	Y	Bone
B10	N	Male	50	1	Y	N	Glandular	IV	N	Bone, Liver

in this study. Another one is to evaluate safety and tolerance of PEG-ENDO combined with TC chemotherapy in advanced NSCLC.

Patients and methods

Patient selection

Patients (age of 18-70) with advanced NSCLC diagnosed phase IIIB or IV by histopathology or cytology who had never been treated with TC chemotherapy, and with no contraindication for chemotherapy, and with no history of anti-angiogenesis therapy, were enrolled into this clinical study. But, patients concurrent using of other anti-cancer drugs or with allergic history to PEG-ENDO (or biological agents) were rejected to the trialists. Patients with severe cardiopulmonary disease, uncontrolled brain metastasis or other malignancy were excluded from the trialists.

25 subjects were assigned to four groups. The detailed patient characteristics of different groups are shown in **Tables 1-4**.

Treatment

PEG-ENDO, PEGylated Recombinant Human Endostatin Solution for Injection, was produced and provided by Protgen Ltd. and met the stringent criteria of biopharmaceuticals for use in human therapy.

Patients received infusion of PEG-ENDO for 120 min weekly and underwent evaluation of vital signs including blood pressure, pulse, respiratory rate, and temperature before treatment, at intervals during infusion, and hourly for 6 h after infusion. After infusion, patients underwent serial pharmacokinetic sampling. All patients were follow-up visited weekly during the screening phase, study phase, and follow-up survery phase underwent evaluation with physical examination including ECOG performance status, vital signs, and laboratory evaluation with complete blood count with manual differential, chemistry evaluation, prothrombin time, and urinalysis. Administration and dosage of TC-PEG-ENDO are shown as **Table 5**.

Response assessment

Objective response was assessed according to the RECIST response criteria: complete response (CR) was complete disappearance of all objective evidence of disease for at least 4 weeks; PR (partial response) was at least 30% reduction in size of measurable lesions without any new lesion for at least 4 weeks; SD (stable disease) was 20% increase or reduction in size of known lesions or appearance of new lesions; PD (progressive disease) was all other situations. The responders included CR and PR patients; correspondingly, non-responders contained SD and PD patients. DCR, referring

Table 5. TC-PEG-ENDO dosage regimen

Drug	Dosage and routes	Timepoint
Paclitaxel	175 (± 20%) mg/m^2, IV (3 h infusion); if body surface area >2 m^2, calculate as 2 m^2	D1, D22
Carboplatin	AUC=5 ± 0.5 mg/ml·min, IV	D2, D23
PEG-ENDO	7.5, 10, 12.5, 15 mg/m^2, IV	D3, D10, D17, D24, D31, D38

Table 6. Baseline demographics and disease characteristics

PEG-ENDO dose	7.5 mg/m^2	10.0 mg/m^2	12.5 mg/m^2	15 mg/m^2
Case number	4	7	4	10
Female/male	4/0	3/4	0/4	2/8
Mean age (year)	59.5	60	59.5	53.8
Smoking	1	4	4	8
Hypertension	1	2	0	1
Stage III/IV	0/4	2/5	0/4	4/6
Retreatment	2	1	0	1
LN	3	7	3	8
Metastasis	4	5	4	6

Table 7. Therapeutic efficacy evaluation

Response assessment	Case number
CR	0/22
PR	5/22
SD	14/22
PD	3/22

disease control rate, include CR, PR and SD patients. Also, time to progress (TTP), referring to the date of diagnosis to the date of disease progression, of all patients was evaluated with univariate and multivariate analyses.

Tolerability and safety

All subjects who were enrolled in the study and received only one dose of PEG-ENDO were included in the safety analyses. Treatment-emergent adverse events, defined as those events that first emerged or worsened during the time when the patient signs informed consent and was included in the study to 1 month after the treatment was completed, were recorded regardless of the relation with the investigational drugs. Serious adverse events were also recorded and were defined as death, initial hospitalization or prolonged hospitalization, life-endangering events (in immediate danger of death), permanent or severe handicapping/functional defects, congenital anormalies/birth defects, what were regarded by the Researcher to be significant due to any reason. Routine laboratory measurements (e.g. haematology and clinical chemistry) and vital signs were measured at screening, baseline and follow-up (~2 weeks after last PEG-ENDO dose).

Results

Patient characteristics

25 subjects fulfilling any of the unresectability criteria were included in the study cohort between December 2012 and September 2014. Patient and tumor characteristics at baseline are summarized in **Table 6**. Most (76% n= 19) patients had lymph node metastases, and only 4 (24%) patients had advanced NSCLC without lymph node metastasis.

Therapeutic efficacy evaluation

25 patients were enrolled into the clinical trial, and 22 subjects completed the programmed two cycles of therapy and were subjected to efficacy evaluation (**Table 7**). The results showed that 5 cases were PR, 14 cases were SD, 3 cases were PD, and the overall DCR (disease control rate) was 86.4%.

Safety and tolerance

Several classical supervision indicators were detected to study the safety of PEG-ENDO-TC regimen therapy, including demographic characteristics, vital signs, physical examination, electrocardiogram, blood in urine routine, liver and kidney function, electrolyte, blood sugar, myocardial enzyme, blood clotting enzyme (**Table 8**).

No serious adverse events were reported in lower dosages of PEG-ENDO (7.5 mg/m^2, 10.0 mg/m^2, 12.5 mg/m^2) plus TC regimen treated groups during the study. However, there were two SAE cases in the 15.0 mg/m^2 PEG-ENDO group, one level 3 kidney injury and one level 4 liver injury. Other subjects of adverse events were mostly reversible. Agranulocytosis

Table 8. Adverse event case number

Supervision indicators	7.5 mg/ m^2 (n=4)	10.0 mg/ m^2 (n=7)	12.5 mg/ m^2 (n=4)	15.0 mg/ m^2 (n=10)
Vital signs	1	1	1	2
Height and weight	0	0	0	0
Physique	1	2	1	2
Electrocardiogram	3	6	2	6
Blood routine	4	7	3	10
Liver function	1	4	2	5
Renal function	0	0	0	2
Electrolyte	2	6	4	6
Urine routines	1	4	4	6
Cardiac function of enzyme	0	0	0	0
Thrombin	0	2	1	2

Table 9. Major adverse events

Type	Case number	CTCAE grade (case number)
Fever	5	I
Elevation of blood pressure	1	I
Agranulocytosis	22	I (1), II (6), III (6), IV (9)
Thrombocytopenia	2	II (1), IV (1)
Anemia	8	I (7), II (1)
Transaminase elevation	7	I (6), IV (1)
Elevated bilirubin	5	I
Creatinine increased	2	I (1), III (1)
Low electrolyte	18	I
Myocardial ischemia	6	I
Sinus speed	9	I
Sinus arrhythmia	2	I
IRBBB	3	I
Electrocardiogram ST-T change	4	I
Proteinuria	9	I
Leucocyturia	5	I

and low electrolyte were the most commonly reported adverse events, occurring 100% (n= 22) and 81.8% (n=18), respectively. The detailed major adverse events were shown in **Table 9**.

Discussion

Angiogenesis offers an attractive target for anti-tumor drug development. A number of endogenous factors, monoclonal antibodies, and synthetic organic molecules with anti-angiogenic activity have been discovered during the past two decades. Endostatin, an endogenous mammalian protein, inhibits the migration and proliferation of endothelial cells and angiogenesis *in vitro* and *in vivo*, which is thought to be an appropriate drug candidate. In clinical setting, ZBP-Endostatin in combination with chemotherapy (NP regimen: NVB+DDP) prolonged the time to tumor progression (TTP) (6.3 months vs. 3.6 months, P=0.0000), improved response rate (RR) (35.4% vs. 19.5%, P=0.0003) and clinical benefit rate (CBR) (73.3% vs. 64.0%, P=0.035) with a favorable toxic profile in advanced NSCLC in Phase II and phase III clinical trials in China [22, 30]. Therefore, in 2005, ZBP-Endostatin was approved by SFDA (trade name: Endostar).

However, as the first generation of Endostatin, ZBP-Endostatin faces many challenges that need to be improved. The biggest one among them is that ZBP-Endostatin needs daily infusion, which increases patients' mental and financial burden. To improve the clinical properties of Endostatin, we have generated PEG-ENDO, the N-terminal mono-PEGylated rh-Endostatin, the purity and biochemical characteristics of which have been intensively investigated and met the stringent criteria of biopharmaceuticals for use in human therapy. PEG-ENDO is much more stable in circulation system, thus it can be used as weekly infusion. The purpose of this Phase I clinical trial is to evaluate safety and tolerance of PEG-ENDO combination with TC regimen therapy in advanced NSCLC and to evaluate of the combination anti-cancer therapeutic efficacy.

In conclusion, PEG-ENDO combination with TC chemotherapy has a beneficial effect for advanced NSCLC patients (PR 22.7%, DCR 84.6%). The overall DCR of combination therapy is higher than that of chemotherapy alone [21]. Besides, there were no clinically significant toxicities associated with the administra-

tion of PEG-ENDO. It is relatively safe and well-tolerated at lower doses, although there may be some risks at a higher dose (15.0 mg/m^2). It will be more comprehensive to evaluate the function of anti-angiogenesis in the treatment of NSCLC by adding some new indicators.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Zhihai Han, Department of Respiratory Disease, The Navy General Hospital of PLA, #6 Fucheng Road, Haidian District, Beijing 100048, China. Tel: +86-10-18600317513; Fax: +86-10-66957586; E-mail: maliehehe@126.com

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