RESEARCH COMMUNICATION

Efficacy and Safety of Endostar Combined with Chemotherapy in Patients with Advanced Solid Tumors

Yan Li[1,2], Xin-En Huang[1,2*], Peng-Wei Yan[1,2], Yong Jiang[1,2], Jin Xiang[3]

Abstract

Purpose: Endostar® is a proteolytic fragment of collagen XVIII that has been shown to have antitumor activity, with a favorable toxicological profile. We conducted this study to investigate its efficacy and safety when combined with chemotherapy in patients with advanced solid tumors. Methods: From July 2006 to September 2008, 45 patients with histologically or cytologically confirmed solid tumors were enrolled into this study. All received Endostar at a dose of 7.5 mg/m^2 /day as an intravenous infusion for more than 7 days, in combination with chemotherapy. Patients were treated until tumor progression or unacceptable toxicity. Results: No treatment related death occurred in this study. Main reported toxicities included: mylosuppression (82.2%), hepatic impairment (42.2%), anorexia (20.0%), nausea (24.4%), vomiting (22.2%), diarrhea (20.0%), febrile (20.0%) and fatigue (24.4%). No complete response was observed. Two patients (2/42) had partial response, twenty-one (21/42) remained stable, and nineteen (19/42) had progressive disease. Median time to tumor progression was 3.0 months (range, 0.5-12.0). Median overall survival was 30.0 months (95% confidence interval: 20.0-40.0) and 1 year survival rate was 81.0%. Conclusion: Our study revealed that toxicity of Endostar combined with chemotherapy in the treatment of solid tumors was tolerable with moderate efficacy.

Keywords: Endostar - chemotherapy - solid tumors - efficacy - safety

Asian Pacific J Cancer Prev, **11**, 1119-1123

Introduction

Endostar®, a novel Recombinant Human Endostatin (rh-Endo) purified from Escherichia coli with an additional nine-amino acid sequence and forming another histidine tag (his-tag) structure, was approved by the State Food and Drug Administration of China (SFDA) in 2005 for the treatment of non-small-cell lung cancer (NSCLC) (Ling et al., 2007). Compared with rh-Endo reported in the literature, the additional nine-amino acid-sequence (MGGSHHHHH) was added at the N-terminal of the protein, which resulted in the formation of a six-His-tag (Song et al., 2005). This His-tag can be chelated with metal ions such as Ni^{2+} (nickel) with a relatively high affinity, and could be utilized to simplify purification and to improve stability of the protein (Song et al., 2005).

In vitro Endostar suppressed MDA-MB-435 cell adhesion to the fibronectin-coated substrate in a concentration-dependent manner, and it could inhibit the wound healing migration of MDA-MB-435 cells and invasion of this cell line through reconstituted extracellular matrix (ECM), probably by decreasing the secretion of Matrix Metalloproteinase-2 (MMP-2) and MMP-9 (Lu et al., 2008). Endostar suppressed the VEGF-stimulated proliferation, migration, and tube formation of human umbilical vein endothelial cells (HUVECs) (Ling et al., 2007; Wu et al., 2008). Endostar induced apoptotic effects in HUVECs through activation of caspase-3 and decrease of Bcl-2 (Ling et al., 2009). Endostar blocked microvessel sprouting from rat aortic rings, inhibited the formation of new capillaries from pre-existing vessels in the chicken chorioallantoic membrane assay (Ling et al., 2007), and affected the growth of vessels in tumor (Ling et al., 2007).

In vivo Endostar affected the growth of endometriotic tissues and the proliferation of breast carcinoma by inhibiting angiogenesis, reducing the expression of vascular endothelial growth factor (VEGF), and the tumor itself (Jiang et al., 2007). In mouse model Endostar inhibited liver metastases from colorectal cancer (Zhou et al., 2006), and had inhibition effect on human lung adenocancer (Ma et al., 2008). The amount of activated circulating endothelial cells decreased significantly in NSCLC patients who responded to Endostar based chemotherapy, increased in those with progressive disease (Wang et al., 2008).

The mechanisms of anti-cancer effect have not yet been characterized fully, but the main mechanisms of of Endostar could include: (a) interactions with adenosine triphosphate (ATP) enzymes on the surface of endothelial cells; (b) blockage of intergrins; (c) and blockage of activities of MMP-2 and MMP-9 (Xu et al., 2007).

In clinical setting, Phase I and Phase II studies

*[1]Department of Chemotherapy, [3]Department of Research Jiangsu Cancer Hospital andß Research Institute, [2]Nanjing Medical University, Nanjing, China. *For correspondence: huangxinen06@yahoo.com.cn*

revealed that Endostar was effective as single agent with good tolerance in pretreated advanced NSCLC patients at the dose of 7.5 mg/m^2 daily (Yang et al., 2004; Yang et al., 2006). Endostar in combination with chemotherapy (NP regimen: NVB+DDP) prolonged the time to tumor progression (TTP) (Shi et al., 2004; Wang et al., 2005; Yang et al., 2005), improved response rate (RR) (Wang et al., 2005) and clinical benefit rate (CBR) (Wang et al., 2005; Shi et al., 2004) with a favorable toxic profile in advanced NSCLC in Phase II and phase III clinical trial in China (Shi et al., 2004; Wang et al., 2005; Yang et al., 2005).

However, most clinical studies on Endostar were confined to patients with NSCLC, with which it was effective and safe (Yang et al., 2004; Shi et al., 2004; Yang et al., 2005; Wang et al., 2005; Yang et al., 2006). Few reports concern Endostar combined with different chemotherapy regimens (except NP) or used for other solid tumors. It is not clear whether Endostar in combination with different chemotherapy regimens could be effective and safe in treating other types of solid tumors, which is the purpose of this study.

Patients and Methods

Patient Eligibility

Eligibility criteria included: patients with histologically or cytologically confirmed solid tumors; age > 18 years; The Karnofsky Performance Score (KPS)>70; life expectancy > 3 months; no psychiatric disorders; with adequate bone marrow function (WBC count >4.0×10^8/dL, platelet count >100×10^8/dL, and hemoglobin >9.0 g/dL), adequate hepatic function (total bilirubin value <1.5 times the upper limit of normal(ULN), serum glutamic pyruvic transaminase (ALT) level <2.0 times ULN, serum glutamic oxaloacetic transaminase (AST) <2.0 times ULN, alkaline phosphatase < 5.0 times ULN, and adequate renal function (serum creatinine <1.5mg/dL). Exclusion criteria included: pregnancy, or lactation; myocardial infarction or angina pectoris within 6 months; radio- or chemotherapy within 4 weeks; major surgery within 2 weeks before study; with uncontrolled congestive heart failure, active infection, or bleeding disorder. All patients provided an informed consent before treatment.

Treatment Schedule

Endostar was administered at a dose of 7.5 mg/m^2 once daily by intravenous infusion for more than 7 days combined with chemotherapy regimens. Completion of more than 7 days of Endostar without breaks was considered one cycle of therapy.

Patient Evaluation

Response was classified according to the WHO criteria (Gehan et al., 2000) with computed tomography or magnetic resonance scan at baseline and every two cycles by investigators. Toxicity was defined according to National Cancer Institution Toxicity Criteria. During each treatment cycle, following laboratory tests on hematologic, hepatic, and renal function were performed. Patients were observed for survival every 2 months until death, or study closure.

Results

Patient Characteristics

Forty-five patients (34 men and 11 women) entered into this study: 28 (62.2%) with lung, 2 (4.4%) with oesophagus, 5 (11.1%) with gastric, 5 (11.1%) with colorectal, 3 (6.6%) with breast, 1 (2.2%) with bladder and 1 (2.2%) with cervical cancer. Base line characteristics of those patients are shown in Table 1. Median age was 56 years (range 31-83 years). Median KPS was 80 (range 70-100). The median time from diagnosis was 7 months (range 0-60 months). More than 90% of patients had stage III or stage IV disease, and the majority had received prior chemotherapy (71.1%) or radiotherapy (33.3%).

In this study, 20 patients (44.4%) received Endostar plus docetaxel or paclitaxel based, 11(24.4%) patients received gemcitabine based, and 7(15.6%) patients received irinotecan (CPT-11) based multiagent chemotherapy (Table 2).

Safety

All 45 patients were assessable for toxicity. Main toxic effects are shown in Table 3. The most common toxicity was myelosuppression: 23 (51.1%) patients with grade 1 or 2, 8 (17.8%) with grade 3 or 4 leukopenia; 10 (22.2%) patients with grade 1 or 2, 6 (13.3%) with grade 3 or 4 anemia; 8 (17.8%) patients with grade 1 or 2, 5 (11.1%) with grade 3 or 4 thrombocytopenia. Grade 1 or 2 elevation of ALT was detected in 9 (20.0%) patients, grade 3 or 4 in 2 (4.4%); elevation of AST, GGT, ALP, or bilirubin was not common. Other treatment related toxicities included: abdominal pain, dizziness, rash, phlebitis, constipation, dental ulcer, alopecia, deep venous thrombosis of the lower extremities anorexia, but in less

Table 1. Patients' Characteristics (Jiangsu Cancer Hospital & Research Institute, Department of Chemotherapy, from July 2006 to September 2008)

Characteristics	No.	%
Gender		
Men	34	75.6
Women	11	24.4
Site of cancer		
Lung	28	62.2
Oesophagus	2	4.4
Gastric	5	11.1
Colorectal	5	11.1
Breast	3	6.7
Bladder	1	2.2
Cervical	1	2.2
Clinical stage		
I	3	6.7
II	1	2.2
III	10	22.2
IV	31	68.9
Prior treatment		
Surgery	30	66.7
Chemotherapy	32	71.1
Radiotherapy	15	33.3

No., number of patients.

Table 2. Regimens of Chemotherapy Combined with Endostar (Jiangsu Cancer Hospital & Research Institute, Department of Chemotherapy, from July 2006 to September 2008)

	Regimen	Dose	Days	No.
1	Docetaxel + Carboplatin	40-60 mg days 1,8; 500 mg day 1.	21	5
2	Docetaxel + Oxaliplatin	40-60 mg days 1,8; 100-150 mg day 1.	21	3
3	Docetaxel + Cisplatin	40-60 mg days 1,8; 20 mg days 1-5 or 40 mg days1-3.	21	2
4	Docetaxel + Nedaplatin	40-60 mg days 1,8; 30 mg days 1-5 or 140 mg day 1.	21	2
5	CPT-11 + Cisplatin	100 mg days1,8; 20 mg days1-5.	21	3
6	CPT-11 + Leucovorin+ 5-FU	200 mg day1; 300mg day1,46-hour; 400 mg /m^2 bolus days1,2 then 600 mg /m^2days1,2 .	14	2
7	Gemcitabine + Carboplatin	1000 mg / m^2 days1,8; 500 mg day 1.	21	4

Table 3. Toxicities of Endostar Combined with Chemotherapy (Jiangsu Cancer Hospital & Research Institute, Department of Chemotherapy, from July 2006 to September 2008)

Toxicities	No. (%)		No. (%)
	Grade 1or 2	Grade 3 or 4	All Grades
Myelosuppression			
Leukopenia	23 (51.1)	8 (17.8)	31 (68.9)
Anemia	10 (22.2)	6 (13.3)	16 (35.5)
Thrombocytopenia	8 (17.8)	5 (11.1)	13 (28.9)
Hepatic enzymes			
ALT	9 (20.0)	2 (4.4)	11 (24.4)
AST	5 (11.1)	2 (4.4)	7 (15.6)
GGT	2 (4.4)	3 (6.7)	5 (11.1)
ALP	0 (0)	1 (2.2)	1 (2.2)
Serum Bilirubin	6 (13.3)	0 (0)	6 (13.3)
Anorexia	8 (17.8)	1 (2.2)	9 (20.0)
Nausea	11 (24.4)	0 (0)	11 (24.4)
Vomiting	8 (17.8)	2 (4.4)	10 (22.2)
Diarrhea	7 (15.6)	2 (4.4)	9 (20.0)
Febrile	9 (20.0)	0 (0)	9 (20.0)
Fatigue	10 (22.2)	1 (2.2)	11 (24.4)

No., number of patients; ALT, SGPT, serum glutamic pyruvictransaminase; AST, SGOT, serum glutamic oxaloacetic transaminase; GGT, γ-Glutamyl transpeptidase; ALP, alkaline phosphatase; Bilirubin, hyperbilirubinemia.

than 10% of our patients. One patient experienced a decrease of SPO2 (transcutaneous oxygen saturation) and developed cyanosis, but recovered after oxygen inhalation and discontinuation of chemotherapy.

Efficacy

Forty-two patients were assessable for response. Two patients (5%) had partial response(PR), 21 (50%) had stable disease(SD) and 19 (45%) experienced disease progression(PD) (Table 4).The median TTP was 3.0 months (range,0.5 to 12.0 months), and median overall survival (OS) was 30.0 months (95% confidence interval, 20.0 to 40.0 months).One year survival rate was 81.0%.

Table 4. Clinical Response of Endostar Combined with Chemotherapy (Jiangsu Cancer Hospital & Research Institute, Department of Chemotherapy, from July 2006 to September 2008)

Results	
Response, No. (%)	
PR	2 (5)
SD	21(50)
PD	19(45)
CBR	23(55)
TTP, months	
Median	3.0
Range	0.5—12.0
OS, months	
Median	30.0
95% CI	20.0-40.0
1 year survival rate, No. (%)	34(81.0)

No., number of patients; PR, partial response; SD, stable disease; PD, progressive disease; CBR, clinical benefit rate, CBR = complete response + PR+SD; TTP, time to tumor progression; OS, overall survival; CI, confidence interval

Discussion

Endostatin was first identified in the conditioned media of murine hemangioendothelioma cells as an antiangiogenic molecule in 1997, with the ability to specifically inhibit endothelial proliferation and potently inhibit angiogenesis and tumor growth (Boehm et al., 1997; O'Reilly et al., 1997). Repeated administration of Endostatin did not produce drug resistance or apparent toxicity (O'Reilly et al., 1996; Boehm et al., 1997; O'Reilly et al., 1997). In 1999, a soluble rh-Endo was produced with characteristics of the primitive Endostatin (Sim et al., 1999). In phase I and phase II clinical trials on single rh-Endo administration, no significant tumor regression was observed in patients with advanced tumors, although rh-Endo was associated with minimal toxicity (Eder et al., 2002; Herbst et al., 2002; Thomas et al.,2003; Hansma et al.,2005; Kulke et al., 2006). The failure of rh-Endo to induce high levels of tumor cell death may explain its lack of significant clinical activity (Davis et al., 2004).

In phase I clinical trial, Endostar was administered as a single agent, 7.5 mg/m^2, 15 mg/m^2, or 30 mg/m^2 daily for 28 days to treat pretreated advanced NSCLC, and the most common toxicities reported were fever and cardiac adverse reactions, including sinus arrhythmia, paroxysmal supraventricular tachycardia, ventricular premature beat, T wave changes shown in electrocardiogram (Yang et al., 2004). In phase II clinical trial on single Endostar administration, decreased hemoglobin and elevation of ALT was observed with cardiac toxicities similar to that in phase I trial (Yang et al., 2006), but the reason for cardiac toxicity was not clear (Yang et al., 2004).

Endostar in combination with NP regimen showed a favorable toxic profile in advanced NSCLC patients (Shi et al., 2004; Wang et al., 2005; Yang et al., 2005; Huang et al., 2008). And there were no significant differences in the incidence of hematotoxicity and nonhematologic toxicity

between study and control groups (Shi et al., 2004; Wang et al., 2005; Yang et al., 2005).

In our study, one of the 3 inassessable patients experienced a decrease of SPO2 and developed cyanosis after an hour of Endostar therapy. Four patients exhibited transient mild elevation of blood pressure (net changes in systolic pressure, 15 to 21 mmHg), and 5 mild decreased blood pressure (net changes in systolic pressure, 13 to 27 mmHg). The other toxicities could be related to the chemotherapy drugs. The results showed that the most common toxicities were grade 1 or 2. In this study, 91% of all the 45 patients had stage III or stage IV tumors, and the physical condition of the patients could be affected by prior systemic chemotherapy or prior radiotherapy. Patient selection may explain the increased incidence of grade 3 or 4 toxicities in patients receiving Endostar combined with chemotherapies.

Because many of angiogenesis inhibitors are growth-inhibiting molecules that work against the tumor vasculature, single agents will have little effect on tumor size in advanced disease (Herbst et al., 2001). Several investigators have suggested that angiogenesis inhibitors may be cytostatic rather than cytotoxic (Shaheen et al., 1999). But most of angiogenesis inhibitors work synergistically with chemotherapy and/or radiotherapy (Herbst et al., 2001). In our study, 2(5%) of 42 patients had partial response, 21(50%) had stable disease and 19 patients (45%) experienced disease progression. But 15 patients experienced disease progression and 12 patients experienced stable disease before study enrollment, which may explain the relatively high incidence of PD and SD observed in this study. The results of this study demonstrate that modest antitumor activity was observed after the treatment of different tumors with Endostar administered as intravenous infusion in combination with chemotherapy.

In conclusion, our preliminary study suggests modest anti-cancer effects and mild toxicity in patients with advanced tumors treated with Endostar and chemotherapy combination.

Acknowledgment

Dr. Xin-En Huang is supported in part by a grant for "333 High-level personnel training project" from Jiangsu Provincial Government, in part by the Kanglaite clinical research fund from Jiangsu Provincial Government (P200908), and in part from a special research fund of Jiangsu Cancer Hospital & Research Institute (ZK200805).

References

Boehm T, Folkman J, Browder T, et al (1997). Antiangiogenic therapy of experimental cancer does not induce acquired drug resistance. *Nature*, **390**, 404-7.

Davis DW, Shen Y, Mullani NA, et al (2004). Quantitative analysis of biomarkers defines an optimal biological dose for recombinant human endostatin in primary human tumors. *Clin Cancer Res*, **10**, 33-42.

Eder JP Jr, Supko JG, Clark JW, et al (2002). Phase I clinical trial of recombinant human endostatin administered as a short intravenous infusion repeated daily. *J Clin Oncol*, **20**, 3772-84.

Gehan EA, Tefft MC (2000). Will There Be Resistance to the RECIST (Response Evaluation Criteria in Solid Tumors)? *J Natl Cancer Inst*, **92**, 179-81.

Hansma AH, Broxterman HJ, van der Horst I, et al (2005). Recombinant human endostatin administered as a 28-day continuous intravenous infusion, followed by daily subcutaneous injections: a phase I and pharmacokinetic study in patients with advanced cancer. *Ann Oncol*, **16**, 1695–701.

Herbst RS, Hess KR, Tran HT, et al (2002). Phase I study of recombinant human endostatin in patients with advanced solid tumors. *J Clin Oncol*, **20**, 3792-803.

Herbst RS, Lee AT, Tran HT, et al (2001). Clinical studies of angiogenesis inhibitors: The University of Texas MD Anderson Center Trial of human endostatin. *Curr Oncol Rep*, **3**, 131-40.

Herbst RS, Mullani NA, Davis DW, et al (2002). Development of biologic markers of response and assessment of antiangiogenic activity in a clinical trial of human recombinant endostatin. *J Clin Oncol*, **20**, 3804-14.

Huang C, Li K (2008). Clinical analysis of advanced NSCLC treated by endostar in combination with NP. *Zhonghua Zhong Liu Fang Zhi Za Zhi*, **15**, 283-5.

Jiang HQ, Li YL, Zou J (2007). Effect of recombinant human endostatin on endometriosis in mice. *Chin Med J*, **120**, 1241-6.

Kulke MH, Bergsland EK, Ryan DP, et al (2006). Phase II study of recombinant human endostatin in patients with advanced neuroendocrine tumors. *J Clin Oncol*, **24**, 3555-61.

Ling Y, Yang Y, Lu N, et al (2007). Endostar, a novel recombinant human endostatin, exerts antiangiogenic effect via blocking VEGF-induced tyrosine phosphorylation of KDR/Flk-1 of endothelial cells. *Biochem Biophys Res Commun*, **361**, 79-84.

Ling Y, Lu N, Gao Y, et al (2009). Endostar induces apoptotic effects in HUVECs through activation of caspase-3 and decrease of Bcl-2. *Anticancer Res*, **29**, 411-8.

Lu N, Ling Y, Gao Y, et al (2008). Endostar suppresses invasion through downregulating the expression of matrix metalloproteinase-2/9 in MDA-MB-435 human breast cancer cells. *Exp Biol Med*, **233**,1013-20.

Ma CJ, Li YQ, Liu CY, et al (2008). The combination of tea polyphenols and rcombinant hman endostatin injection (Endostar) inhibits xenografted tumor growth of human lung adenocarcinoma A549. *Zhongguo Zhong Yi Yao Xin Xi Za Zhi*, **15**, 41-3.

O'Reilly MS, Holmgren L, Chen C, et al (1996). Angiostatin induces and sustains dormancy of human primary tumors in mice. *Nat Med*, **2**, 689-92.

O'Reilly MS, Boehm T, Shing Y, et al(1997). Endostatin: An endogenous inhibitor of angiogenesis and tumor growth. *Cell*, **88**, 277-85.

Shaheen RM, Davis DW, Liu W, et al (1999). Antiangiogenic therapy targeting the tyrosine kinase receptor for vascular endothelial growth factor inhibits the growth of colon cancer liver metastases and induces tumor and endothelial cell apoptosis. *Cancer Res*, **59**, 5412-6.

Shi HL, Xu LY, Liu Z (2004). Phase II clinical trial of homemade human rh-endostatin in the treatment of patients with stage IIIB- IVnon-small cell lung cancer. *Zhongguo Fei Ai Za Zhi*, **7**, 325-8.

Song HF, Liu XW, Zhang HN, et al (2005). Pharmacokinetics of His-tag recombinant human endostatin in Rhesus monkeys. *Acta Pharmacol Sin* , **26**, 124-8.

Sim BK, Fogler WE, Zhou XH, et al (1999). Zinc ligand-

disrupted recombinant human Endostatin: Potent inhibition of tumor growth, safety and pharmacokinetic profile. *Angiogenesis*, **3**, 41-51.

Thomas JP, Arzoomanian RZ, Albierti D, et al (2003). Phase I pharmacokinetic and pharmacodynamic study of recombinant human endostatin in patients with advanced solid tumors. *J Clin Oncol*, **21**, 223-31.

Wang JW, Sun Y, Liu YY, et al (2005). Results of phase III trial of rh-endostatin (YH-16) in advanced non-small cell lung cancer (NSCLC) patients. *Zhongguo Fei Ai Za Zhi*, **8**, 283-90.

Wang J, Huang C, Wei XY, et al (2008). Changes of activated circulating endothelial cells and survivin in patients with non-small cell lung cancer after antiangiogenesis therapy. *Chin Med J*, **121**, 2234-40.

Wu G, Zhang RG, Ren JH, et al (2008). Autophagic cell death of human hepatoma cells induced by Endostar, a recombinant human endostatin. *Cancer Biother Radiopharm*, **23**, 735-40.

Xu FG, Ma QY, Sha HC (2007). Optimizing drug delivery for enhancing therapeutic efficacy of recombinant human endostatin in cancer treatment. *Crit Rev Ther Drug Carrier Syst*, **24**, 445-92.

Yang L, Wang JW, Tang ZM, et al (2004). A phase I clinical trial for recombinant human endostatin. *Zhongguo Xin Yao Za Zhi*, **13**, 548-53.

Yang L, Wang JW, Cui CX, et al (2005). Rh-endostatin (YH-16) in combination with vinorelbine and cisplatin for advanced non-small cell lung cancer: a multicenter phase II trial. *Zhongguo Xin Yao Za Zhi*, **14**, 204-7.

Yang L, Wang JW, Sun Y, et al (2006). Randomized phase II trial on escalated doses of Rh-endostatin (YH-16) for advanced non-small cell lung cancer. *Zhonghua Zhong Liu Za Zhi*, **28**, 138-41.

Zhou ZW, Wan DS, Wang GQ, et al (2006). Inhibitory effect of angiogenesis inhibitor YH-16 on liver metastases from colorectal cancer. *Chin J Cancer*, **25**, 818-22.