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Tolerance and Pharmacokinetics of Recombinant Human Endostatin Administered as Single-Dose or Multiple-Dose Infusions in Patients With Advanced Solid Tumors: A Phase I Clinical Trial

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Abstract

Objective: This study aimed to investigate the tolerance and pharmacokinetic characteristics of recombinant human endostatin (rh-endostatin) administered as single-dose or multiple-dose infusions in patients with advanced solid tumors. **Methods**: This phase I trial was designed as a single-center, single-arm, nonrandomized, open-label, dose-escalation study. The trial consisted of 2 parts: a single-dose part and a multiple-dose part, each with 3 dose comparison groups. Rh-endostatin was administered as an intravenous injection only once at a dose of 5 mg/m^2, 7.5 mg/m^2, or 10 mg/m^2 in the single-dose part and as a daily intravenous injection for 14 days at the same doses in the multiple-dose part. The serum pharmacokinetics, toxicity and immunogenicity of rh-endostatin were evaluated. **Results**: Dose-limiting toxicity (DLT) was not observed in any group. A few patients developed cardiotoxicity, such as QT prolongation or narrow arrhythmia. Other adverse events were slight coagulation abnormalities and haematological abnormalities. For rh-endostatin doses of 5 mg/m^2, 7.5 mg/m^2, and 10 mg/m^2, the mean C_{max} values in the single-dose part were 344 ± 38.7 ng/mL, 524 ± 157 ng/mL, and 800 ± 201 ng/mL, respectively, and the average AUC_{0-t} values were 3290 ± 3790 ng•h/mL, 4940 ± 4380 ng•h/mL, and 5050 ± 3980 ng•h/mL, respectively. The $C_{max\ ss}$ values of the 3 doses in the multiple-dose part were 575 ± 270 ng/mL, 531 ± 106 ng/mL, and 864 ± 166 ng/mL, respectively, and the $AUC_{0-\tau}$ values were 3610 ± 1040 ng•h/mL, 3290 ± 1090 ng•h/mL, and 5180 ± 1210 ng•h/mL, respectively. The C_{max} of a single-dose regimen showed linear kinetic characteristics. The patients in the single-dose group were negative for serum antibodies against rh-endostatin, while one patient in the multiple-dose group was positive. **Conclusions**: Rh-endostatin as a daily intravenous injection for 14 days in patients with advanced solid tumors is safe and well tolerated, without DLT, at doses of 5 mg/m^2, 7.5 mg/m^2, and 10 mg/m^2. Serum antibodies against rh-endostatin were very low after multiple infusions. For phase II trials, the recommended rh-endostatin dose is 10 mg/m^2 as a daily intravenous injection for 14 days.

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Keywords
angiogenesis, rh-endostatin, phase I clinical trial, pharmacokinetics dose-limiting toxicity

Abbreviations
rh-endostatin, recombinant human endostatin; DLT, Dose-limiting toxicity; FDA, Food and Drug Administration; SAEs, serious adverse events; LLOQ, lower limit of quantification; RC_{max}, the mean cumulative ratio; ADA, Anti-drug Antibody; MMP-2, matrix metalloproteinase-2; ECLA, electrochemiluminescence assay; VEGF, vascular endothelial growth factor

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Introduction

For solid tumors larger than 2 mm^3, growth and metastasis depend on neovascularization,[1] with such tumors relying on neovascularization to provide metabolically necessary substances to support growth.[2] tumor neovascularization is a complex process regulated by multiple factors, and blocking angiogenesis by inhibiting the expression of promotive factors has become an important strategy for antitumor therapy.[3,4]

Endostatin is an endogenous anti-angiogenic factor that was first isolated from mouse vascular endothelial cell tumor cultures by O'Reilly in 1996.[5] It is a carboxy-terminal-hydrolysed fragment derived from collagen XVIII that has a molecular weight of 20 kDa and contains 184 amino acids. Endostatin inhibits tumor angiogenesis and growth by blocking the migration, proliferation, and lumen formation of vascular endothelial cells.[6]

A previous report showed that recombinant human endostatin (rh-endostatin) prepared using *Escherichia coli* or *Pichia* as the expression system exhibits significant antitumor activity in vitro and in animal experiments.[7] In October 1998, intravenous injection of rh-endostatin produced by EntreMed was approved by the Food and Drug Administration for phase I clinical trials. These trials showed that the drug was well tolerated and there was no dose-limiting toxicity (DLT), even at the maximum dose of 240 mg/m^2.[8–11] In 2002, a phase II clinical trial of rh-endostatin was conducted, and the results showed that no patient achieved a complete or partial response.[12] Given the unsatisfactory results of the study and high production cost of rh-endostatin, the progress of rh-endostatin towards becoming an antitumor treatment in the phase II clinical trial was halted.

A new rh-endostatin drug for administration by injection was used in the current study; this formulation was developed by Suzhou Zhongkai Biopharmaceutical Factory and the Institute of Biomedical Research, Jiangsu Wuzhong Pharmaceutical Group Co. This rh-endostatin drug was prepared using an *E. coli* expression system, has a molecular weight of 20 kDa and contains 184 amino acids, 2 pairs of disulfide bonds, no glycosylation site, and the same amino acid sequence as the natural human endothelial inhibitor, without any modification or reconstruction. The results of an evaluation over the long term in preclinical studies showed that this drug is different from the previous products due to the expression vector and expression strain used to generate the drug. In this phase I trial, we aimed to evaluate tolerance and the pharmacokinetics of rh-endostatin after single and multiple intravenous injections of the drug.

Materials and Methods

Patient Selection

The Tianjin Medical University Cancer Institute and Hospital ethics committee approved this study on August 24, 2016, and the approval number is E2016138.

Each included patient signed an written informed consent form prior to enrolment, which included a description of the study drug and study procedures, as well as the patient's privacy, risks, benefits and compensation. The enrolled patients met the following inclusion criteria: aged 18 to 70 years, BMI of 19 to 28 kg/m^2, ECOG status of 0 to 2, expected survival time ≥3 months, pathologically confirmed advanced solid tumors refractory to standard therapy or for which there was no standard therapy, and adequate organ function. Adequate organ function was defined as a thrombin time and prothrombin time of 1.5 × the upper limit of normal, without a bleeding tendency or thrombosis; an absolute neutrophil count $\geq 1.5 \times 10^9$/L; a platelet count of $\geq 100.0 \times 10^9$/L; a haemoglobin concentration ≥90.0 g/L; and bilirubin, AST, ALT, and serum creatinine 1.5 × the upper limit of normal.

The exclusion criteria were any previous chemotherapy, biotherapy, immunotherapy or radiotherapy within 4 weeks of enrolment or having undergone any previous major surgery within the previous 3 weeks. Additionally, patients were excluded if they had a history of severe cardiovascular disease, such as uncontrollable hypertension, myocardial infarction, typical angina pectoris, congestive heart failure, severe arrhythmia, prolonged QT interval syndrome, atrioventricular block, cerebral infarction or brain hemorrhage. Patients with severe infection, uncontrolled malignant pleural or abdominal effusion, mental illness, primary or metastatic brain tumor, a history of HIV or chronic hepatitis B or C infection, syphilis, an allergic constitution, or pregnancy and patients who were breastfeeding were also excluded.

In error, we did not prospectively register this trial, but we have now registered it retrospectively at the Research Registry https://www.researchregistry.com/: registration number 7187.

Study Design and Rh-Endostatin Administration

This phase I trial was designed as a single-centre, single-arm, nonrandomized, open-label, dose-escalation study. The trial was divided into 2 parts: a single-dose part and a multiple-dose part. Rh-endostatin was administered as a 120-min intravenous injection only once in the single-dose part and as a daily 120-min intravenous injection for 14 days in the multiple-dose part. Based on the results of preclinical pharmacodynamic, toxicological and pharmacokinetic studies on rh-endostatin, as well as the phase I clinical doses of similar products used domestically and abroad, 3 dose comparison groups, namely, 5 mg/m^2, 7.5 mg/m^2 and 10 mg/m^2, were designed for both parts, and 6 to 8 patients with advanced solid tumors were allocated to each dose comparison group. Any adverse events that occurred during the clinical trial were recorded according to CTCAE 4.03. If there was no DLT in 1/3 or more of the subjects enrolled in a dose group, the next highest dose comparison group was tested; if DLT was observed, dose escalation was ended.

Sample Preparation and Pharmacokinetic Assessment

For the single-dose part of the study, blood was collected before and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 48, and 72 h after dosing (measured from the start of the intravenous injection). For the multiple-dose part, blood was collected at the following time points: before and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 h after dosing on day 1, before dosing on days 12, 13, and 14, and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 48 and 72 h after dosing on day 14. Three milliliters of whole blood was collected at each time point.

After the blood had clotted, each sample was centrifuged at 2000 rpm and 4 °C for 10 min, and at least 0.6 mL of the resulting serum was transferred to a cryogenic tube for testing, with the remaining serum being transferred to a back-up tube. The samples were stored upright at −40 °C until use and were sent to the laboratory for determination of rh-endostatin concentrations by electrochemiluminescence assay.

The following relevant single-dose pharmacokinetic parameters were measured: $t_{1/2\beta}$, MRT, AUC_{0-t}, $AUC_{0-\tau}$, $AUC_{0-\infty}$, Vz, CL, C_{max}, and T_{max}, among others. The pharmacokinetic parameters for the multiple-dose part of the study were $t_{1/2\beta\ ss}$, $AUC_{0-\tau}$, $AUC_{0-t\ ss}$, $AUC_{0-\infty\ ss}$, $V_{z\ ss}$, CL_{ss}, $C_{max\ ss}$, $C_{min\ ss}$, and $T_{max\ ss}$, among others.

Immunogenicity Assessment

To assess the immunogenicity of rh-endostatin, detection of antibodies against rh-endostatin was performed. Blood samples (3 mL) were collected before the first dose and on day 7 ± 1 of the single-dose part or on day 21 ± 1 of the multiple-dose part. After the blood had clotted, the samples were centrifuged at 2000 rpm and 4 °C for 10 min. At least 0.8 mL of the resulting serum was transferred to a cryogenic tube for testing, and the remaining serum was transferred to a back-up tube. Anti-rh-endostatin antibodies in serum were detected using the Bridging-ECL method.

Statistical Methods

WinNonlin 6.3 was used for the pharmacokinetic analyses. No comparative statistics were used. Data are presented in a descriptive manner. Descriptive statistics were used in this study. Mean and standard deviations were used for Continuous data. The frequency and percentage are provided for count data.

Results

Patient Characteristics and Treatments

From December 2016 to December 2017, 24 patients with advanced solid tumors were enrolled in the single-dose part, and 25 patients were enrolled in the multiple-dose part (Table 1).

All subjects in the single-dose part completed the entire study according to the protocol. One subject in the 5-mg/m^2 dose group and 2 in the 10-mg/m^2 dose group were excluded

Table 1. Patient Characteristics.

	Single dose (mg/m^2)			Multiple doses (mg/m^2)		
	5	7.5	10	5	7.5	10
Enrolled patients, n	7	9	8	9	8	8
Median age (years) (Min, Max)	62.0 (46, 68)	53.0 (41, 66)	52.5 (46, 62)	57.0 (46, 68)	56.0 (41, 66)	52.0 (46, 62)
Male, n	2	2	1	2	2	1
Female, n	5	7	7	7	6	7
Median BMI (kg/m^2) (Min, Max)	23.2 (19.3, 26.5)	24.0 (19.6, 27.8)	25.0 (20.7, 27.9)	23.6 (19.3, 28.0)	25.0 (19.6, 27.8)	25.0 (20.7, 27.9)
Breast cancer, n	4	6	7	6	5	7
Lung cancer, n	2	2	1	2	2	1
Ovarian cancer, n	1	1	0	1	1	0

from the pharmacokinetic analysis because blood was drawn from the infusion side of the limb, which might have affected the drug concentration in these samples. In the multiple-dose part, 3 patients withdrew from the study. Among these 3 subjects, 1 in the 5-mg/m^2 dose group experienced grade 1 haemoptysis during the trial and, given the mechanism of action of the study drug, withdrew from the trial due to the risk of adverse event exacerbation. This participant had advanced central lung cancer, and the adverse event in question was considered to be due to the disease itself and might not have been related to rh-endostatin. The second subject, who was also in the 5-mg/m^2 dose group, withdrew from the trial due to grade 1 palpitations that were unlikely to be related to rh-endostatin treatment according to the researcher's judgement. The third subject, who was in the 10-mg/m^2 dose group, withdrew for personal reasons. After all exclusions, 22 subjects remained in the multiple-dose part and were included in the pharmacokinetic analysis.

Toxicities

Overall, rh-endostatin treatment was well tolerated, with only grade 1 to 2 drug-related adverse events in the single-dose part (Table 2). There were no DLTs or drug-related serious adverse events (SAEs). The grade 2 drug-related adverse events observed were a prolonged QT interval on ECG (1 patient in the 5-mg/m^2 group, 1 patient in the 7.5-mg/m^2 group, and 3 patients in the 10-mg/m^2 group), anemia (1 patient in the 10-mg/m^2 group), and neutropenia (1 patient in the 10-mg/m^2 group).

Additionally, no DLTs or drug-related SAEs occurred in the multiple-dose group (Table 2). All drug-related adverse events were grade 1 to 2, except for 2 grade 3 adverse events in the 5 mg/m^2 dose group (1 patient with increased ALT and AST), which were considered to possibly be related to the study drug. Twenty-three grade 2 drug-related adverse events occurred during the study, the most common of which was a prolonged QT interval on ECG.

Pharmacokinetics

The pharmacokinetic parameters of the subjects in the single-dose part are shown in Table 3, and time curves for the mean rh-endostatin blood concentration are shown in Figure 1. In all dose groups, rh-endostatin blood concentrations increased gradually with time after dosing; the C_{max} was reached at approximately 2.00 h after dosing in most subjects, followed by a slow decrease in the blood concentration, which generally fell back to below the assayed lower limit of quantification (LLOQ) within 12 h after dosing (78.13 ng/mL). The C_{max} and AUC_{0-t} displayed a linear pharmacokinetic profile with increasing dose administration, as illustrated in Figure 1.

The pharmacokinetic parameters of the subjects in the multiple-dose part are shown in Table 3, and the mean blood concentration-time curves are shown in Figure 2. The steady-state T_{max} was approximately 2 h after dosing, and the blood

Table 2. Drug-related Adverse Events in the Single-dose and Multiple-dose Parts.

	Single dose				Multiple doses			
AE	5 mg/m^2 n (%)	7.5 mg/m^2 n (%)	10 mg/m^2 n (%)	Total n (%)	5 mg/m^2 n (%)	7.5 mg/m^2 n (%)	10 mg/m^2 n (%)	Total n (%)
QT interval prolonged	1 (14.3)	3 (33.3)	4 (50.0)	8 (33.3)	2 (22.2)	3 (37.5)	3 (37.5)	8 (32.0)
QT interval shortened	1 (14.3)	2 (22.2)	0	3 (12.5)	0	3 (37.5)	1 (12.5)	4 (16.0)
ST-T change on ECG	1 (14.3)	4 (44.4)	0	5 (20.8)	3 (33.3)	3 (37.5)	0	6 (24.0)
Arrhythmia	1 (14.3)	2 (22.2)	0	3 (12.5)	0	1 (12.5)	1 (12.5)	2 (4.0)
Bradycardia	1 (14.3)	1 (11.1)	0	2 (8.3)	2 (22.2)	1	0	3 (12.0)
Tachycardia	1 (14.3)	0	0	1 (4.2)	2 (22.2)	0	0	2 (8.0)
Hypotension	2 (28.6)	1 (11.1)	0	3 (12.5)	-	-	-	-
Hypertension	-	-	-	-	1 (11.1)	1 (12.5)	1 (12.5)	3 (12.0)
Thrombin time prolonged	1 (14.3)	3 (33.3)	2 (25.0)	6 (25.0)	0	2 (25.0)	2 (25.0)	4 (16.0)
Prothrombin time prolonged	-	-	-	-	1 (11.1)	0	0	1 (4.0)
APTT shortened	0	2 (22.2)	3 (37.5)	5 (20.8)	0	3 (37.5)	5 (62.5)	8 (32.0)
D-Dimer increased	0	1 (11.1)	0	1 (4.2)	2 (22.2)	1 (12.5)	2 (25.0)	5 (20.0)
Fibrinogen increased	1 (14.3)	0	0	1 (4.2)	1 (11.1)	1 (12.5)	0	2 (8.0)
ALT increased	0	0	1 (12.5)	1 (4.2)	1 (11.1)	0	2 (25.0)	3 (12.0)
AST increased	-	-	-	-	1 (11.1)	0	1 (12.5)	2 (8.0)
GGT increased	-	-	-	-	1 (11.1)	0	2 (25.0)	3 (12.0)
Bilirubin increased	-	-	-	-	1 (11.1)	0	2 (25.0)	3 (12.0)
Anemia	3 (42.9)	1 (11.1)	1 (12.5)	5 (20.8)	2 (22.2)	2 (25.0)	1 (12.5)	5 (20.0)
Neutropenia	1 (14.3)	1 (11.1)	1 (12.5)	3 (12.5)	1 (11.1)	0	1 (12.5)	2 (8.0)
Fatigue	0	0	1 (12.5)	1 (4.2)	-	-	-	-

concentration fell back to below the LLOQ (78.13 ng/mL) approximately 12 h after completion of the first dose infusion. The trough concentrations of the drug were around the LLOQ on day 12, day 13 or day 14 after dosing.

Compared with the first-dose C_{max} and AUC_{0-24} values, the $C_{max\ ss}$ and $AUC_{0-\tau}$ values were elevated in the 5-mg/m^2 group after multiple infusions (Figure 3). For 5, 7.5, and 10 mg/m^2, the mean cumulative ratio (R_{Cmax}) values were 1.61 ± 0.631, 1.12 ± 0.175, and 1.11 ± 0.132, respectively, and the R_{AUC} values were 2.28 ± 0.743, 1.04 ± 0.357, and 1.20 ± 0.358, respectively, indicating that a 14-day infusion with a concentration of 7.5 mg/m^2 or 10 mg/m^2 did not result in significant accumulation in the subjects but that repeated administration at 5 mg/m^2 may result in some accumulation. The confidence interval method was used to evaluate the pharmacokinetic profile after multiple dosing, and neither the trend in $C_{max\ ss}$ nor that in $AUC_{0-\tau}$ was characterized by linear pharmacokinetics in the dose range of 5 to 10 mg/m^2 .

Anti-drug Antibody (ADA) Assays

All the subjects tested negative for ADAs before dosing and during the follow-up period in each single-dose comparison group. Among the multiple-dose comparison groups, only 1 sample from the 10-mg/m^2 group was positive for ADAs.

Discussion

Endothelial inhibitors are pan-targeted endogenous broad-spectrum anti-angiogenic factors with multiple mechanisms of action. These mechanisms include actions on vascular endothelial growth factor (VEGF) receptors to prevent VEGF binding to endothelial cells; downregulation of angiogenic factors, blocking degradation of the basal lamina by matrix metalloproteinase-2 (MMP-2), inhibiting endothelial cell proliferation and migration, and promoting endothelial cell apoptosis.[13] The recombinant human vascular endothelial inhibitor Endostar has the advantages of a broad antitumor spectrum, mild toxicity, and less resistance to continuous medication; it is widely used to treat non-small-cell lung cancer, colorectal cancer, breast cancer, malignant melanoma and other solid tumors and can be used to control malignant effusion.[14–20]

Three dose groups, namely, 5, 7.5, and 10 mg/m^2, were designed for this study. In the current trial, patients in each dose group were able to tolerate the single dose as well as multiple doses, and no DLTs or drug-related SAEs were observed.

The incidences of cardiotoxicity with rh-endostatin treatment reported in the literature range from 6.4% to 13.2%, and these toxicities include mainly myocardial ischemia, electrocardiographic abnormalities and proiosystole.[21] In the phase I clinical trial of Endostar reported by YANG Lin et al., the main adverse events were arrhythmia, paroxysmal supraventricular tachycardia, ventricular proiosystole, and abnormal ECG T waves.[22] Additionally, the adverse cardiovascular events in 61 patients with non-small-cell lung cancer treated with Endostar reported by Wenjing MENG et al. included ECG abnormalities, D-dimer anomalies, platelet abnormalities, and blood pressure fluctuations.[23] Among these adverse events, ECG abnormalities had the highest incidence, and older patients were more likely to have ECG abnormalities and blood pressure

Table 3. Pharmacokinetic Parameters in the Single-dose Part (mean ± SD) and Multiple-dose Part (mean ± SD).

Parameter	5 mg/m^2	7.5 mg/m^2	10 mg/m^2
Single-dose Part (mean ± SD)			
T_{max} (h)	1.92 ± 0.2	1.89 ± 0.2	1.92 ± 0.2
C_{max} (ng/mL)	344 ± 38.7	524 ± 157	800 ± 201
AUC_{0-t} (ng•h/mL)	3290 ± 3790	4940 ± 4380	5050 ± 3980
$t_{1/2}$ (h)	-[a]	1.53 ± 0.51	3.08 ± -[a]
MRT_{0-t} (h)	10.8 ± 15.1	13.7 ± 14.5	9.52 ± 11.8
V_z (L/m^2)	-[a]	12.0 ± 2.15	15.0 ± -[a]
CL (L/h/m^2)	-[a]	5.58 ± 0.90	3.38 ± -[a]
Multiple-dose Part (mean ± SD)			
Day 1 T_{max} (h)	2.00 ± 0	1.81 ± 0.26	2.00 ± 0
Day 1 C_{max} (ng/mL)	378 ± 70.1	484 ± 102	774 ± 138
Day 1 AUC_{0-t} (ng•h/mL)	1530 ± 873	2880 ± 1930	4180 ± 2190
Day 1 AUC_{0-24} (ng•h/mL)	2420 ± 692	3620 ± 1670	4670 ± 1900
Day 1 MRT_{0-t} (h)	2.62 ± 2.42	5.56 ± 4.77	5.21 ± 4.58
Day 14 T_{max} (h)	314 ± 0.41	314 ± 0.18	314 ± 0
Day 14 $C_{min\ ss}$ (ng/mL)	45.2 ± 50.6	37.8 ± 52.9	86.9 ± 49.8
Day 14 $C_{max\ ss}$ (ng/mL)	575 ± 270	531 ± 106	864 ± 166
Day 14 $C_{av\ ss}$ (ng/mL)	150 ± 43.2	137 ± 45.6	216 ± 50.5
Day 14 $AUC_{0-\tau}$ (ng•h/mL)	3610 ± 1040	3290 ± 1090	5180 ± 1210
Day 14 AUC_{0-t} (ng•h/mL)	6020 ± 3060	5500 ± 4000	9530 ± 3520

Note: [a] Concentration data were not available for this time point because more than 1/3 of the individual data points were below the LLOQ. The parameters $AUC_{0-\infty}$, R^2_{-a}, λ_z, $t_{1/2}$, $AUC_{\%Extrap}$, $V_{z\ ss}$, and CL_{ss} were not included in the descriptive statistics because the linear regression fit R^2_{-a} of <0.800 or $AUC_{\%Extrap}$ of > 20% of the elimination phase did not meet the statistical requirements.

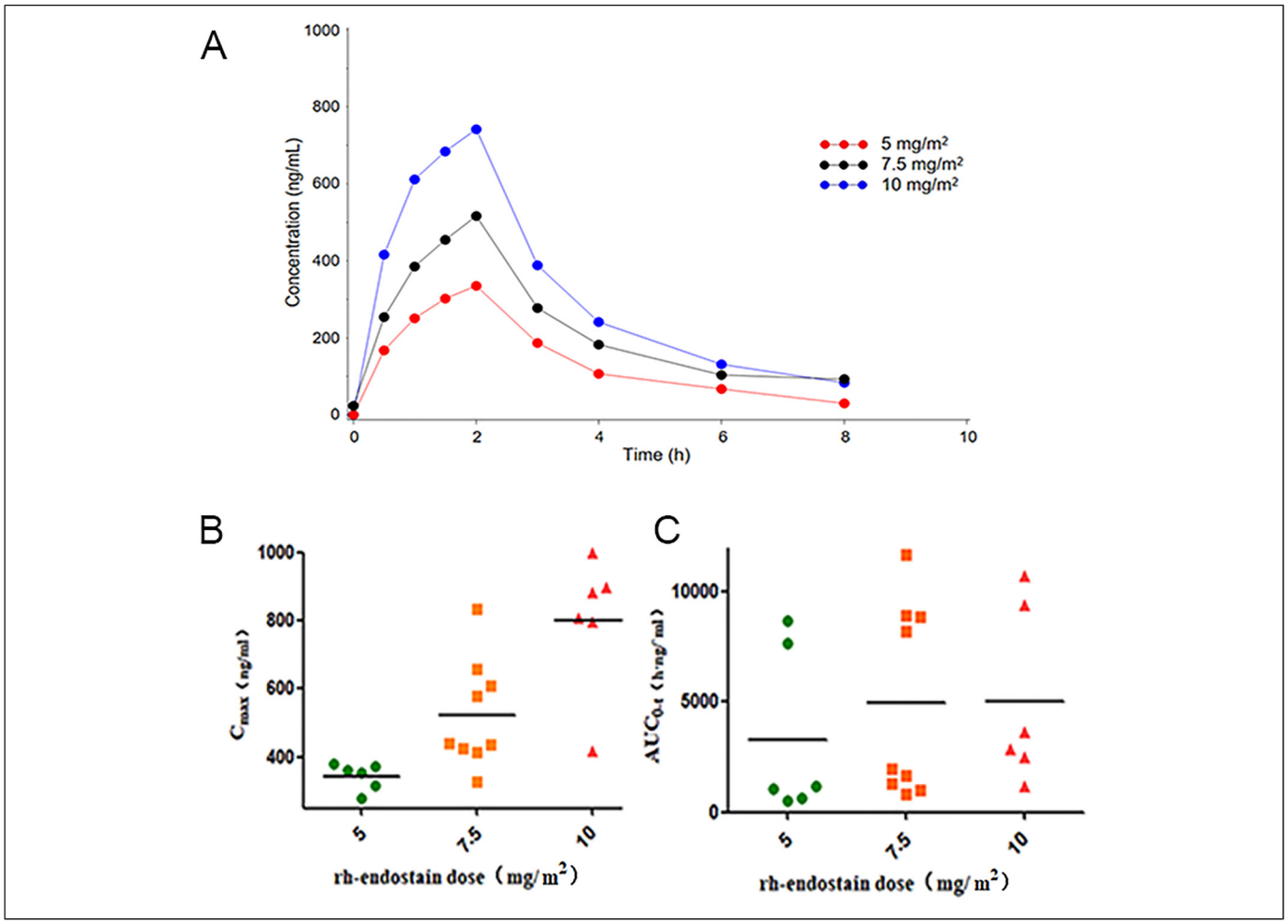

Figure 1. The 8-h linear curve showing the mean blood concentration of rh-endostatin over time in the single-dose part of the study (A); the C_{max} (B) and AUC0-t (C) in each single-dose group.

fluctuations than younger patients. Nevertheless, all of these adverse events improved after treatment ended.

The mechanism of rh-endostatin cardiotoxicity is likely to be related to cardiomyocyte apoptosis and angiogenesis inhibition. The former is an important mode of cardiomyocyte death, and studies have shown that cardiomyocyte apoptosis plays an important role in the pathophysiology of various cardiovascular diseases and the development of cardiotoxicity for many drugs.[24–26] Cardiomyocyte apoptosis has multiple triggers, such as excessive cardiac anterior and posterior loads, calcium overload in cardiomyocytes, myocardial ischemia, and hypoxia. Qin Jing et al.[27] found significant pathological changes in the rat myocardium after high-dose (12 mg/kg) Endostar administration from 4 weeks to 8 weeks, involving enlarged myocardial cell gaps, disruptions in the myocardial cell arrangement, localized myocardial cell nuclear lysis, subepicardial inflammatory cell infiltration, and blurred myocardial cell structure. Structural damage to the mitochondria and significant expansion of the sarcoplasmic reticulum in cardiomyocytes were also observed by electron microscopy, suggesting that mitochondrial pathway-induced myocardial apoptosis may be an important mechanism of Endostar-induced cardiotoxicity. Cardiomyocytes are terminally differentiated cells. Thus, as apoptosis increases, the number of active cardiomyocytes decreases, which in turn leads to impairment of cardiac function and induces various cardiovascular events, such as heart failure, arrhythmia, and myocardial ischemia. In addition, as an angiogenesis inhibitor, rh-endostatin acts on the VEGF receptor cell signaling pathway, blocking autophosphorylation of VEGF receptors and activation of certain protein kinases to inhibit neovascularization, which may cause cardiac ischemia and hypoxia and induce a variety of cardiotoxicities.

In this trial, the observed adverse events included mainly various arrhythmias and ECG, blood pressure, coagulation, and hematological or liver function abnormalities, the majority of which were grade 1 to 2; indeed, the only drug-related grade 3 adverse event was elevated ALT and AST. A detailed assessment of a patient's history of cardiovascular disease prior to treatment, selection of an appropriate treatment population, close monitoring of cardiac function during treatment, and avoidance of treatment in combination with cardiotoxic drugs will help to prevent cardiovascular events.

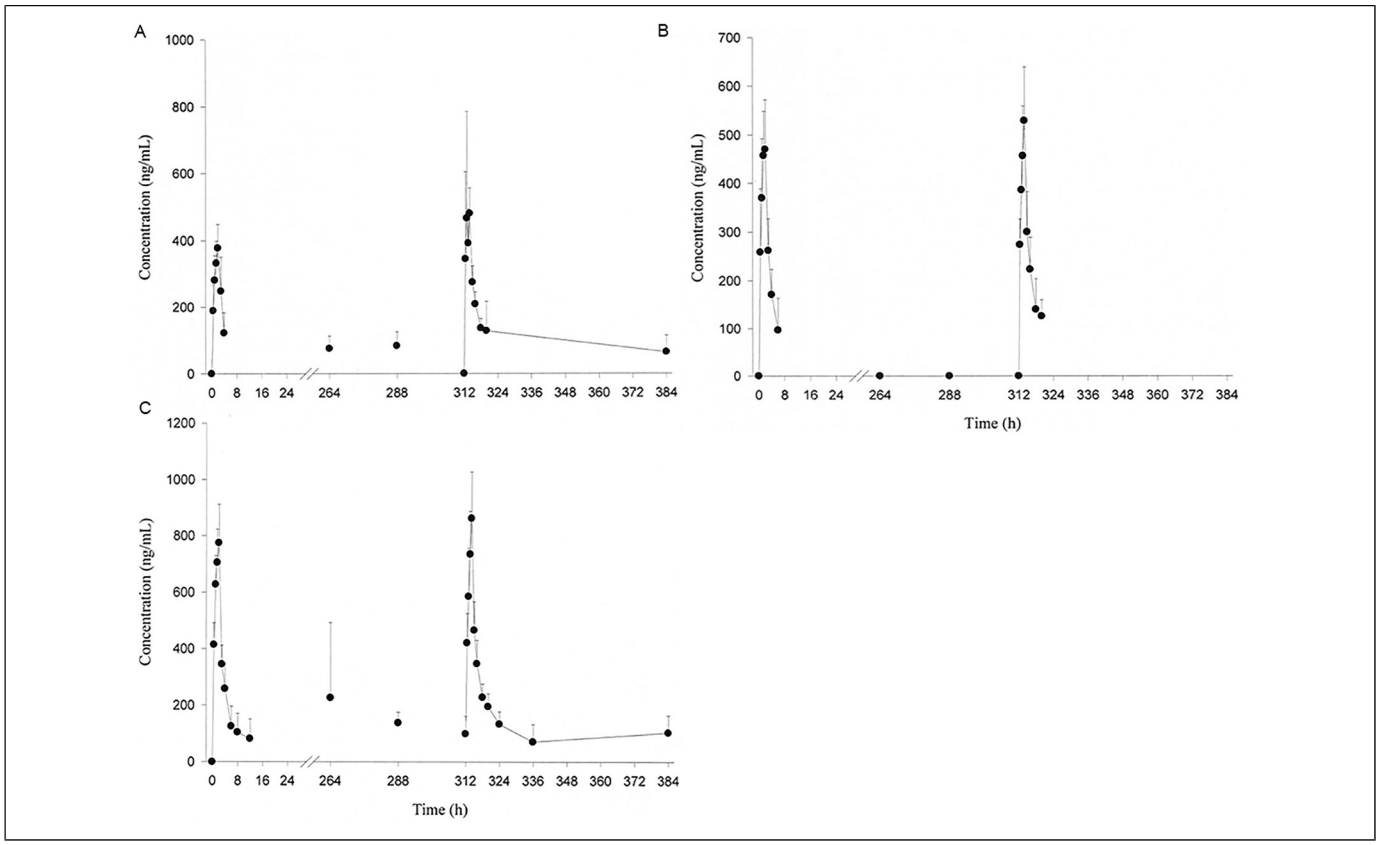

Figure 2. Mean blood concentration-time curves in the 5-mg/m2 group (A), 7.5-mg/m^2 group (B), and 10-mg/m^2 group (C) in the multiple-dose part of the study.

Kisker et al. showed that rh-endostatin has a tumor-shrinking effect at steady-state blood concentrations of 200 to 300 ng/mL after application.[28] After daily administration at 15 mg/m^2, the $C_{min\ ss}$ of the rh-endostatin produced by EntreMed, USA, was 48 ± 25 ng/mL, the $C_{max\ ss}$ was 394 ± 55 ng/mL, and the AUC_{0-24} was 31 ± 15 μg•min/mL.[8] Bi Hu et al. showed that the blood concentration of rh-endostatin increased gradually over time after a daily intravenous drip at 10 mg/m^2, and the peak blood concentration was generally within 2 h. The blood concentration decreased immediately after the end of dosing and basically returned to the pre-dosing level within 12 h after the completion of dosing. After 28 days of dosing, the $C_{min\ ss}$ was 166.31 ± 53.36 ng/mL, the $C_{max\ ss}$ was 861.29 ± 160.25 ng/mL, and the AUC_{0-24} was 40.32 ± 8.56 μg•h/

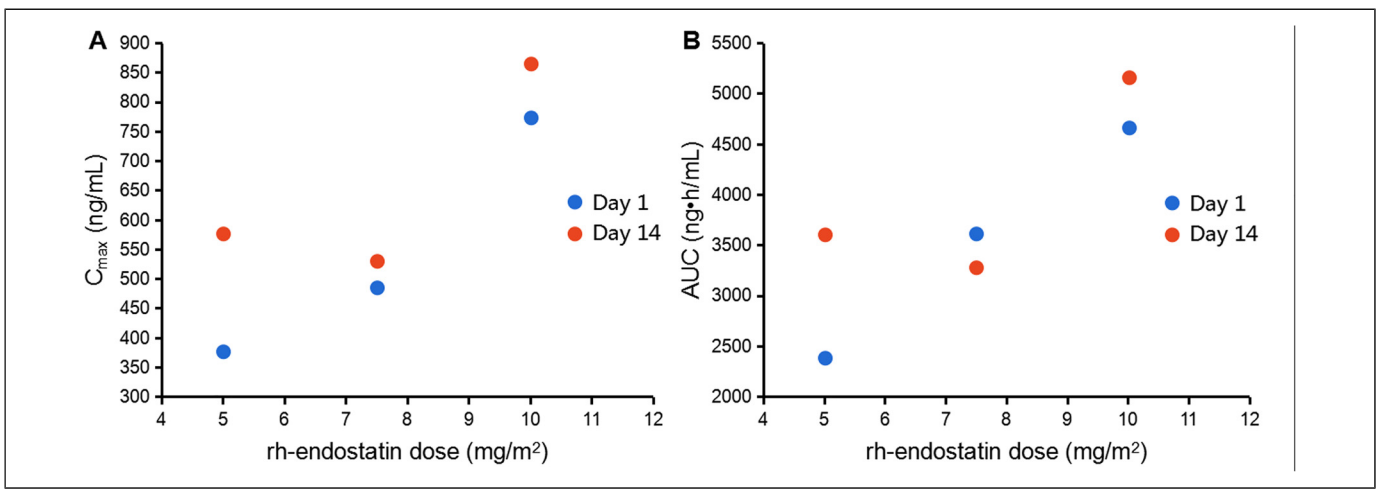

Figure 3. C_{max} (A) and AUC (B) on day 1 and day 14 in each multiple-dose group.

mL.[29] The phase I clinical trial by YANG Lin et al. showed that after 28 days of intravenous dosing with Endostar at 7.5 mg/m^2 daily, the $C_{min\ ss}$ was 220 ± 120 ng/mL, the $C_{max\ ss}$ was 870 ± 470 ng/mL, and the AUC_{0-24} was 2660 ± 1760 ng•h/mL. In this study, after 14 days of 7.5 mg/m^2 daily infusion, the $C_{min\ ss}$ was 37.8 ± 52.9 ng/mL, the $C_{max\ ss}$ was 531 ± 106 ng/mL, and the AUC_{0-24} 3620 ± 1670 ng•h/mL; however, after 14 days of daily infusion with 10 mg/m^2 rh-endostatin, the $C_{min\ ss}$ was 86.9 ± 49.8 ng/mL, the $C_{max\ ss}$ was 864 ± 166 ng/mL, and the AUC_{0-24} was 4670 ± 1900 ng•h/mL.[22] The pharmacokinetic characteristics of the drug after continuous administration were similar to those of Endostar and other rh-endostatin intravenous injections, and the $C_{min\ ss}$ and $C_{max\ ss}$ in the same dose group were slightly lower than those of Endostar, but they were above the blood concentration for effective tumor suppression. As rh-endostatin is a protein-based biological agent, its biological activity requires correct folding into a specific 3-dimensional structure.[30] This may explain the differences in the pharmacokinetic parameters of drugs of the same class, such those described above, and these discrepancies may be related to differences in the production process, molecular structure, stability, purity, and effective folding.

There is a very important relationship between changes in the conformation of drug molecules and their biological activity, which is due to the fact that the interaction between a drug and a receptor requires complementarity in the conformation of both. If the conformation of a drug molecule changes, the physicochemical properties and charge density of the drug molecule will change, which in turn will affect the binding of the drug to the receptor, the absorption and transport of the drug in the body, and ultimately affect the therapeutic effect of the drug, and even have unexpected adverse events. The rh-endostatin formulation prepared by Jiangsu Wuzhong Pharmaceutical Group Co., Ltd with E. coli as the expression system and used in this study has a higher purity than the similar product prepared by EntreMed, Inc. using Pichia as the expression system. And it has the same amino acid sequence as the natural human endothelial inhibitor, without any modification or reconstruction. Therefore, the dose administered in the current study was only approximately one-tenth that of EndreMed.

The following were calculated in this study: mean cumulative ratio R_{Cmax} of 1.61 ± 0.631 and R_{AUC} of 2.28 ± 0.743 after multiple doses of 5 mg/m^2; R_{Cmax} of 1.12 ± 0.175 and R_{AUC} of 1.04 ± 0.357 after multiple doses of 7.5 mg/m^2; R_{Cmax} of 1.11 ± 0.132 and R_{AUC} of 1.20 ± 0.358 after multiple doses of 10 mg/m^2. Based on R_{Cmax} results for each dose group, it was inferred that no significant accumulation occurred in the subjects after 2 h of daily administration of rh-endostatin at 7.5 mg/m^2 or 10 mg/m^2 for 14 days, though some accumulation appeared to have occurred in the 5 mg/m^2 group.

In this study, only 3 dose groups were preset. Although no DLTs or drug-related SAEs occurred, this study drug did not continue to climb to higher doses, which was due to previously obtained preclinical pharmacodynamic, toxicological and clinical pharmacokinetic results. According to the results of similar drug studies, it was essentially determined that this study drug had reached a therapeutically effective dose. In addition, no other adverse events such as fever, rash, and diarrhea, which occurred with similar products, were observed in this study.[21] It may be due to the small number of patients enrolled, so the sample size needs to be expanded in the follow-up research for continued observation.

It was reported that 60.0% of the phase I studies provided no statistical justification of the expansion cohort sample sizes. The average sample size of a phase I study has increased from 33.8 patients to 73.1 patients from 1988 to 2012.[31] However, it is a limitation that we did not perform a sample size calculation because this trial was designed as a phase I study. There are 49 patients enrolled in this clinical trial. In the future study, we will recommend that phase I studies justify their planned sample sizes so that any predefined expectation of benefit is stated a priori and provide unambiguous rules determining study completion and success.

Conclusions

This study showed that rh-endostatin injection has good safety and tolerability in all dose groups when administered as a single dose or daily for 14 days. A phase II clinical trial at a dose of 10 mg/m^2 is recommended to further evaluate the efficacy and safety of this drug in oncology patients.

Authors Contribution

XW and YS wrote the manuscript; XW, YS, and YJ treated patients and/or gathered data. YZC and ZT designed the study; XW, YZC, and WZ evaluated the data; all authors critically read and contributed to the manuscript; all authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

Each included patient signed an informed consent form prior to enrollment. The Tianjin Medical University Cancer Institute and Hospital ethics committee approval number is E2016138. This study was registered retrospectively at the Research Registry https://www.researchregistry.com/ with Registration number 7187.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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