ORIGINAL ARTICLE

A phase I, single and continuous dose administration study on the safety, tolerability, and pharmacokinetics of neorudin, a novel recombinant anticoagulant protein, in healthy subjects

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Abstract

The aim of this study was to evaluate the tolerability, safety, and pharmacokinetics of single and continuous dose administration of recombinant neorudin (EPR-hirudin, EH) by intravenous administration in healthy subjects, and to provide a safe dosage range for phase II clinical research. Forty-four subjects received EH as a single dose of between 0.2 and 2.0 mg/kg by intravenous bolus and drip infusion. In addition, 18 healthy subjects were randomly divided into three dose groups (0.15, 0.30, and 0.45 mg/kg/h) with 6 subjects in each group for the continuous administration trial. Single or continuous doses of neorudin were generally well tolerated by healthy adult subjects. There were no serious adverse events (SAEs), and all adverse events (AEs) were mild to moderate. Moreover, no subjects withdrew from the trial because of AEs. There were no clinically relevant changes in physical examination results, clinical chemistry, urinalysis, or vital signs. The incidence of adverse events was not significantly related to drug dose or systemic exposure. After single-dose and continuous administration, the serum EH concentration reached its peak at 5 min, and the exposure increased with the increase in the administered dose. The mean half-life ($T_{1/2}$), clearance (Cl), and apparent volume of distribution (Vd) of EH ranged from 1.7 to 2.5 h, 123.9 to 179.7 ml/h/kg, and 402.7 to 615.2 ml/kg, respectively. The demonstrated safety, tolerability, and pharmacokinetic characteristics of EH can be used to guide rational drug dosing and choose therapeutic regimens in subsequent clinical studies.

Clinical trial registration: Chinadrugtrials.org identifier: CTR20160444.

KEYWORDS
clinical pharmacology, in vivo, pharmacokinetics, safety pharmacology

1 | INTRODUCTION

The prevalence of thrombus formation is likely to increase in humans because of growing populations and longer life expectancy.[1] Thrombotic events can be potentially life threatening and may prolong the length of hospital stay and result in chronic disability.[2] Thrombosis is the most common underlying pathology of the three major cardiovascular disorders: ischemic heart disease (acute coronary syndrome, ACS), stroke, and venous thromboembolism (VTE).[3] ACS, the acute manifestation of ischemic heart disease, resulting from coronary arterial thrombus formation remains a major cause of morbidity and mortality worldwide.[4] VTE, primarily including pulmonary embolism and deep venous thrombosis, affects an estimated 750,000 people in the United States each year and is the cause of more than 100,000 deaths annually. Of the patients with thromboembolism, 15.4% die within 90 days after the index diagnosis.[5,6]

In general, clinical guidelines for the treatment of VTE recommend subcutaneous low-molecular-weight heparin (LMWH) as well as fondaparinux, followed by a vitamin K antagonist (VKA). Both LMWH and VKAs are associated with a risk of potentially fatal bleeding.[7,8] Unlike heparin, hirudin is an antithrombotic agent produced by the salivary glands of the medicinal leech, Hirudo medicinalis, and can directly act on thrombin and effectively inhibit both free and bound thrombin. In some animal models of deep vein injury, hirudin was shown to be a more effective antithrombotic drug than heparin.[9,10] However, treatment with hirudin increases the risk of systemic bleeding, a main and sometimes lethal adverse effect.[11,12]

Neorudin (EPR-hirudin, EH) is a targeted hirudin variant 2-Lys47 (HV2) fusion protein which is composed of 68 amino acids, and has a theoretical molecular weight of 7284 Da.[13-15] EH was developed as a prodrug of HV2 by introducing EPR (Glu-Pro-Arg), which is recognized and cleaved by FXIa into the N-terminus of HV2.[16] EH exerts antithrombotic effects by releasing its active metabolite, HV2, at the thrombus site *via* FXIa-mediated cleavage of EPR, resulting in direct inhibition of thrombin. However, when intact, EH does not display anticoagulant activity. The construction and mechanism of EH determine that it not only effectively inhibits thrombus formation but also reduces the risk of bleeding by increasing the specificity and efficiency of hirudin.[17,18] EH is a prodrug and has no anticoagulant activity unless it is cleaved by the activated coagulation factor XI at the location of the clot. This phase I study was conducted in healthy subjects and no clots were formed or activation of coagulant factor XI occurred. Therefore, no active hirudin was converted from neorudin, thus, the PD could not be determined in the current study.

Preclinical studies in rat models of thrombus formation have shown that EH is effective and safe for the treatment of thrombosis. Compared with LMWH and hirudin, the bleeding side effects of EH were lower at similar antithrombotic effects.[19] In addition, a repeat-dose toxicity study in cynomolgus monkeys found no adverse effects after repeated intravenous administration of EH. The pharmacokinetic results in rhesus monkeys showed that the half-life ($T_{1/2}$) and Tmax of EH were approximately 1 h and 3 min, respectively, when evaluated using enzyme-linked immunosorbent assay.

The purpose of this first-in-human study was to assess the elementary safety and pharmacokinetics of EH in healthy volunteers.

2 | MATERIALS AND METHODS

2.1 | Subjects

Healthy, non-smoking men or women aged between 18 and 45 years with a body mass index of 19–26 kg/m^2 (weight ≥50 kg) were eligible for inclusion in the study. Health status was determined by a prestudy medical history, physical examination, and clinical laboratory test evaluations. Women who were nursing or pregnant and subjects who were infected with human immunodeficiency virus, hepatitis B and/or hepatitis C viruses, or syphilis were excluded from the study. The subjects were not allowed to smoke cigarettes, and consume alcohol or grapefruit-containing products during screening, admission, and follow-up. Subjects with a history of donating blood or significant blood loss in the past 3 months (≥400 ml) were excluded from the study.

2.2 | Design

A Phase I, single- and continuous dose administration study of safety, tolerability, and pharmacokinetics of EH by intravenous administration in healthy subjects was conducted. The study was sponsored by Beijing SH Biotechnology Co., Ltd. and Beijing Institute of Radiation Medicine, and was performed at the Phase 1 Clinical Research Center of Beijing You'an Hospital, Capital Medical University. Both studies were approved by applicable institutional review boards/ethics committees and conducted in accordance with country regulations, the International Conference on Harmonisation Good Clinical Practice guidelines, and the principles of the Declaration of Helsinki. Written informed consent was obtained from all subjects.

2.2.1 | Determination of the EH dose escalation range

According to the "Guidelines for Estimating the Maximum Recommended Starting Dose of Drugs for the First Clinical Trial of Healthy Adult Volunteers" promulgated by the CENTER FOR DRUG EVALUATION and the No Observed Adverse Effect Level (NOAEL) calculation results of preclinical animal trials, it was known that the most suitable NOAEL of EH from long-term toxicity testing in cynomolgus monkeys was 1.3 mg/kg. The recommended clinical dose of Refludan,[20] a similar drug from Bayer, approved by the FDA on 6th March 1998 for the treatment of heparin-induced thrombocytopenia (HIT), is 0.4 mg/kg by intravenous bolus, and then 0.15 mg/kg/h of continuous intravenous infusion for 2–7 days. Preclinical studies of EH showed that 0.4 mg/kg of hirudin was equivalent to 0.416 mg/kg EH, and the pharmacodynamic dose range from EH animal test

studies was approximately 0.06–2.13 mg/kg. The preclinical mouse and cynomolgus acute toxicity tests of this drug showed that its maximum tolerated dose (MTD) was much higher than the planned clinical effective dose. Consequently, it was considered safe and practical to initially consider a dose increase range 0.2–2.0 mg/kg in this study.

2.2.2 | Single-dose regimen

In the single-dose trial, four subjects were enrolled in the preliminary trial and 40 subjects were enrolled in the formal trial. In the preliminary trial, considering that the test drug was being used for the first time in humans, in order to guarantee the safety of the subjects to the utmost extent, a test dose of 0.2 mg/kg was administered to one healthy subject and the subject observed for 7 days to confirm the safety of EH. Subsequently, the remaining three subjects were re-entered into the trial and observed for 7 days. In the formal trial, the randomized, placebo-controlled, dose-escalating study was divided into five dose groups, with eight volunteers per group (test drug: placebo = 6:2), and the order of dose increase was 0.4, 0.8, 1.2, 1.6, and 2.0 mg/kg per administration. The research center determined the drug grouping information of the subjects according to a randomized system.

2.2.3 | Continuous dose administration

For this trial, three dose open groups for continuous administration were employed, with six volunteers in each group, and the time of continuous administration was 24 h. After drug administration, the subjects were discharged after 2 days of hospitalization, and the trial ended after 28 days. In each dose group, the dose of intravenous bolus (bolus ≥30 s) was 0.4 mg/kg, and the increasing range of the maintenance dose was 0.15, 0.30, and 0.45 mg/kg/h. One subject in each group was first enrolled, and the remaining subjects were then enrolled after observing that there were no safety issues at least 48 h after the end of drug administration. It was confirmed that there were no safety issues after the last subject in the previous dose group had completed the observation for at least 7 days, after which the other subjects were entered into the next dose group. Supervising and guiding the process of drug clinical trials was performed by establishing a data and safety monitoring committee.

2.3 | Safety assessment

The following clinical safety assessments were included in the study: a physical examination, vital signs (height, weight, blood pressure, heart rate, axillary temperature, and respiratory rate), clinical laboratory tests (full blood count, urine routine, blood chemistry, coagulation function, stool routine and occult blood, 12-lead ECG cardiac

monitoring, and chest X-ray examination), and recording of adverse events. Adverse experiences were monitored throughout the study period. The investigators evaluated all clinical adverse events in terms of intensity (mild, moderate, or severe), duration, severity, outcome, and relationship to the study drug.

2.4 | Pharmacokinetic assessments

2.4.1 | Pharmacokinetics of single-dose administration

Forty-four subjects at six dose levels (0.2–2.0 mg/kg) participated in the pharmacokinetic assessment of the single-dose administration study. Serum was collected within 5 min before administration; 5 min, 15 min, and 30 min after the start of administration; and 5 min, 20 min, 40 min, 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 8 h, 12 h, and 24 h after administration to test the blood drug concentration.

2.4.2 | Pharmacokinetics of continuous dose administration

Eighteen subjects in the three dose groups of 0.15, 0.30, and 0.45 mg/kg/h participated in the pharmacokinetics analysis of the continuous dose administration study. Serum was collected within 5 min before administration; 5 min, 30 min, 4 h, 8 h, 12 h, and 24 h after the start of administration; and 5 min, 20 min, 40 min, 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 8 h, 12 h, and 24 h after the end of administration to test the blood drug concentration. In the 0.30 mg/kg/h dose group, plasma was also collected at 5 min, 8 h, and 24 h after the start of administration, and 5 min after the end of administration to test the blood drug concentration. In addition, in the 0.15 and 0.30 mg/kg/h group, urine was collected before administration; 0–4, 4–8, 8–12, 12–16, 16–20, 20–24 h after administration; and 0–4, 4–8, 8–12, 12–16, 16–20, 20–24, 24–32, 32–40, and 40–48 h after the end of the administration period to test the urine drug concentration.

2.5 | Data and statistical analysis

Treatment-emergent adverse events (TEAEs) and adverse reactions in each dose group were summarized according to the System Organ Class (SOC) and Preferred Term (PT). TEAE refers to an adverse event that occurs at or after the first administration of a drug to 28 days (inclusive) after the last administration of the drug. The severity of adverse events was classified using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) software, version 4.03.

For safety data, such as laboratory tests, vital signs, electrocardiograms, and physical examination results, baseline data, post-dose data, and changes from baseline readings were summarized according to each follow-up and each dose group. Regarding

TABLE 1 Summary statistics of demographic and baseline characteristics

Dosage n (%)		Single-dose administration						Continuous dose administration		
		0.2 mg/kg (N = 4)	0.4 mg/kg (N = 8)	0.8 mg/kg (N = 9)	1.2 mg/kg (N = 8)	1.6 mg/kg (N = 8)	2.0 mg/kg (N = 8)	0.15 mg/kg/h (24 h) (N = 6)	0.30 mg/kg/h (24 h) (N = 6)	0.45 mg/kg/h (24 h) (N = 6)
Age (years)	Mean ± SD	28.8 ± 3.69	28.8 ± 6.86	27.4 ± 7.35	29.4 ± 6.37	30.9 ± 5.33	28.0 ± 5.13	30.0 ± 4.29	29.7 ± 7.92	30.5 ± 8.17
	Med	29.0	27.0	27.0	27.0	29.5	27.0	30.5	31.0	31.5
	Q1, Q3	27, 31	25, 31	21, 30	25, 34	27, 33	25, 32	27, 33	24, 36	23, 36
	Min, Max	24, 33	22, 44	19, 40	23, 41	26, 42	21, 36	24, 35	18, 38	20, 41
Gender, n (%)	Male	3 (75.0%)	4 (50.0%)	3 (33.3%)	6 (75.0%)	6 (75.0%)	4 (50.0%)	4 (66.7%)	4 (66.7%)	4 (66.7%)
	Female	1 (25.0%)	4 (50.0%)	6 (66.7%)	2 (25.0%)	2 (25.0%)	4 (50.0%)	2 (33.3%)	2 (33.3%)	2 (33.3%)
Height (cm)	Mean ± SD	171.3 ± 12.04	165.0 ± 7.50	163.7 ± 9.87	167.5 ± 5.32	169.6 ± 7.76	161.6 ± 8.73	166.7 ± 9.1	167.2 ± 3.1	162.3 ± 5.1
	Med	175.5	165.0	163.0	170.0	171.5	164.0	168.5	168.0	162.0
	Q1, Q3	163, 180	160, 170	157, 167	165, 171	163, 175	154, 168	157, 174	164, 169	157, 166
	Min, Max	154, 180	154, 178	151, 182	157, 172	158, 181	148, 173	156, 176	163, 171	157, 170
Weight (kg)	Mean ± SD	63.0 ± 8.87	59.9 ± 7.45	64.1 ± 10.20	61.8 ± 6.16	64.4 ± 8.45	58.5 ± 7.43	64.8 ± 7.73	64.0 ± 5.66	59.8 ± 7.52
	Med	66.0	61.0	65.0	61.5	64.5	56.5	61.5	64.5	58.0
	Q1, Q3	58, 68	53, 66	57, 71	59, 67	57, 72	52, 67	59, 73	59, 69	53, 68
	Min, Max	50, 70	50, 70	51, 83	50, 69	53, 76	50, 68	58, 76	57, 70	53, 69
BMI (kg/m²)	Mean ± SD	21.40 ± 0.829	21.92 ± 1.132	23.81 ± 1.653	21.96 ± 1.537	22.28 ± 1.508	22.35 ± 1.660	23.30 ± 1.519	22.87 ± 1.467	22.65 ± 1.949
	Med	21.45	21.80	23.80	21.80	22.10	22.35	23.80	23.40	22.00
	Q1, Q3	20.8, 22.1	21.1, 22.4	22.8, 25.1	20.6, 23.6	21.2, 22.5	21.5, 23.5	22.2, 24.5	21.9, 23.9	21.5, 23.9
	Min, Max	20.4, 22.3	20.6, 24.2	20.7, 25.7	20.3, 23.6	21.0, 25.7	19.4, 24.7	20.8, 24.7	20.4, 24.2	20.6, 25.9

Abbreviations: BMI, body mass index; Max, maximum; Med median; Min, minimum; SD, standard deviation.

whether the test results were normal or not and whether they were clinically meaningful as classified data, a shift table was used to describe the change from baseline to each follow-up result after administration.

Using the blood/urine PK concentration analysis set, the blood/urine concentration at each planned time point was descriptively summarized, and for each dose group, the concentration-time curve of each subject and the average concentration-time curve of each group were generated.

Pharmacokinetic data were processed and graphed using Microsoft Excel and Origin Pro, version 8.0. Pharmacokinetic parameters were calculated using the non-compartmental model (NCA) of Phoenix 64 (WinNonlin 6.3). Medical history and adverse events were coded using MedDRA, while past and combined medications were coded using the WHO Drug Dictionary.

Statistical analysis was performed for both trials using SAS® software, version 9.4 (SAS Institute Inc.), and a p-value <.05 was considered statistically significant.

3 | RESULTS

3.1 | Subject characteristics

In the single-dose trial, 44 subjects who met the selection criteria received the test agent to obtain safety data and were included in the safety analysis set. In the continuous administration trial, 18 subjects who met the selection criteria received the test agent to obtain safety data and were included in the safety analysis set. Summary demographic information of the 62 subjects who completed the study is summarized in Table 1. Healthy, non-smoking men or women aged between 27.4 and 30.9 years with a body mass index of 21.4–23.81 kg/m^2 were included in the single-dose trial. Healthy, non-smoking men or women aged between 29.7 and 30.5 years with a body mass index of 22.65–23.30 kg/m^2 were included in the continuous dose administration trial.

3.2 | Safety Results

Adverse events (AEs) recorded during the entire study were mild to moderate and well controlled, and most subjects recovered without treatment. There was no statistically significant difference in the incidence of adverse events between the test (20 of 34 subjects) and placebo groups (5 of 10 subjects) in the single-dose study ($p = 0.62$). In total, 51 AEs were reported from 20 of the 34 subjects in the test group, and 14 of them were considered by the investigator to be related to EH (Table 2). The incidence of AEs was notably higher in the continuous dose administration study than in the single-dose study. Sixty-eight AEs were reported from 17 of the 18 subjects in the continuous dose study, and 58 of the 68 reported AEs were considered by the investigator to be related to EH (Table 3). Hypohemoglobinemia was the main AE recorded in the single-dose study, while the increase in D-dimer, reticulocyte, and activated partial thromboplastin time (APTT) were the main AEs reported in the continuous administration study. However, no treatment or dose-related trends in the reported AEs were observed. No serious adverse events (SAEs) were reported; thus, no subjects were withdrawn due to AEs and no AEs led to death.

TABLE 2 Incidence of all adverse events in the single-dose trial

Adverse event	0.2 mg/kg (N = 4)	0.4 mg/kg (N = 8)	0.8 mg/kg (N = 8)	1.2 mg/kg (N = 8)	1.6 mg/kg (N = 8)	2.0 mg/kg (N = 8)
Subjects with at least one adverse reaction	2(50.0), [2]	1(12.5), [1]	2(25), [4]	1(12.5), [2]	3(37.5), [4]	2(25.0), [4]
Summary	2(50.0), [2]	1(12.5), [1]	1(12.5), [2]	1(12.5), [1]	1(12.5), [1]	2(25.0), [4]
Prothrombin time prolongation	0	0	0	0	0	1(12.5), [1]
Prothrombin level decreased	0	0	0	0	0	1(12.5), [1]
Positive occult blood	0	0	1(12.5), [1]	0	0	1(12.5), [1]
Abnormal electrocardiogram T wave	0	0	0	0	0	1(12.5), [1]
APTT prolonged	1(25.0), [1]	0	0	0	0	0
Urinary erythrocyte	1(25.0), [1]	0	1(12.5), [1]	0	0	0
Thrombin time prolongation	0	0	0	1(12.5), [1]	0	0
Abnormal electrocardiogram	0	0	0	0	1(12.5), [1]	0
Fibrinogen decreased	0	1(12.5), [1]	0	0	0	0
Abnormalities of blood and lymphatic system	0	0	2(25), [2]	1(12.5), [1]	3(37.5), [3]	0
Hypohemoglobinemia	0	0	2(25), [2]	1(12.5), [1]	3(37.5), [3]	0

TABLE 3 Incidence of all adverse events in the continuous dose administration trial

Adverse event	0.15 mg/kg/h (24 h) (N = 6)	0.30 mg/kg/h (24 h) (N = 6)	0.45 mg/kg/h (24 h) (N = 6)	Summary (N = 18)
Subjects with at least one adverse reaction	6(100), [18]	5(83.3), [15]	6(100.0), [25]	17(94.4), [58]
Summary	6(100), [14]	5(83.3), [13]	6(100.0), [25]	17(94.4), [52]
White blood cell count increased	0	0	3(50.0), [4]	3(16.7), [4]
Prothrombin time Prolongation	0	1(16.7), [1]	3(50.0), [3]	4(22.2), [4]
Prothrombin level decreased	0	1(16.7), [1]	3(50.0), [3]	4(22.2), [4]
Centriole count increased	0	0	3(50.0), [3]	3(16.7), [3]
APTT prolongation	0	2(33.3), [3]	2(33.3), [3]	4(22.2), [6]
Reticulocyte count increased	2(33.3), [2]	1(16.7), [3]	2(33.3), [2]	5(27.8), [7]
Renal creatinine clearance decreased	0	0	1(16.7), [1]	1(5.6), [1]
Glomerular filtration rate decreased	0	0	1(16.7), [1]	1(5.6), [1]
Fibrin D-dimer increased	3(25), [4]	2(33.3), [2]	1(16.7), [1]	6(33.3), [7]
ECG ST segment depression	0	0	1(16.7), [1]	1(5.6), [1]
Serum bilirubin increased	0	0	1(16.7), [1]	1(5.6), [1]
Serum creatinine increased	0	0	1(16.7), [1]	1(5.6), [1]
Fibrinogen decreased	2(33.3), [3]	0	1(16.7), [1]	3(16.7), [4]
Urinary erythrocyte	1(16.7), [2]	1(16.7), [1]	0	2(11.1), [3]
Thrombin–antithrombin III complex reduced	0	1(16.7), [2]	0	1(5.6), [2]
Fibrin degradation products increased	2(33.3), [3]	0	0	2(11.1), [3]
Metabolic and nutritional diseases	0	2(33.3), [2]	0	2(11.1), [2]
Hypertriglyceridemia	0	2(33.3), [2]	0	2(11.1), [2]
Respiratory system, chest, and mediastinal diseases	1(16.7), [1]	0	0	1(5.6), [1]
Epistaxis	1(16.7), [1]	0	0	1(5.6), [1]
Gastrointestinal diseases	1(16.7), [1]	0	0	1(5.6), [1]
Gingival bleeding	1(16.7), [1]	0	0	1(5.6), [1]
Abnormalities of blood and lymphatic system	2(33.3), [2]	0	0	2(11.1), [2]
Hypohemoglobinemia	2(33.3), [2]	0	0	2(11.1), [2]

3.3 | Pharmacokinetics

Serum or urine samples from all subjects who participated in both studies (62) were collected for pharmacokinetic analysis using an ultra-performance liquid chromatography/tandem mass spectrometry method.[21,22] However, data from 10 subjects in the placebo group did not result in detection of EH during analysis and was therefore ignored.

3.3.1 | Pharmacokinetics after single-dose administration

The mean serum concentration–time data and PK parameters of EH are shown in Table 4 and Figure 1A. Following 5 min of infusion, the serum concentration of EH reached its peak at the end of the infusion period, and the exposure increased with an increase in the dose. The half-life ($T_{1/2}$), clearance (Cl), and volume of distribution (Vd) of each dose group changed in the ranges of 1.7–2.5 h, 123.9–179.7 ml/h/kg, and 402.7–615.2 ml/kg, respectively.

The mean serum concentration of EH gradually increased with increasing dose, and the characteristics of serum concentration–time data in each dose group were similar. With an increase in the administered dose, the exposure of hirudin, the active metabolite of EH, in serum also gradually increased. The trend of EH plasma concentration was similar to that of hirudin, showing a linear relationship. After 7 h of EH administration, the serum hirudin concentration in each dose group was too low to be detected and quantified. The ratio of the area under the curve of hirudin to that of EH was approximately 2.4–6.3% (Table 5).

3.3.2 | Pharmacokinetics after continuous administration

The mean serum concentration–time data and PK parameters of EH are summarized in Table 4 and Figure 1B. The mean serum concentration of EH gradually increased with an increase in dose, and the characteristics of serum concentration–time data in each dose group were similar. Following an increase in the administered dose,

TABLE 4 Pharmacokinetic parameters by dose after single-dose and continuous dose administration

Single dose						
Dosage n (%) Mean ± SD	0.2 mg/kg (N = 4)	0.4 mg/kg (N = 8)	0.8 mg/kg (N = 8)	1.2 mg/kg (N = 8)	1.6 mg/kg (N = 8)	2.0 mg/kg (N = 8)
HL_Lambda_z (h)	1.7 ± 0.3	1.9 ± 0.3	2.3 ± 0.3	2.1 ± 0.1	2.3 ± 0.3	2.2 ± 0.3
Tmax (h)	0.083 ± 0	0.083 ± 0	0.083 ± 0	0.083 ± 0	0.083 ± 0	0.083 ± 0
Cmax (ng/ml)	981.9 ± 148.1	2223.9 ± 488.1	4025.1 ± 1144.6	4389.7 ± 646.3	6524.6 ± 1087.4	9050.3 ± 1958.6
AUC last (h*ng/ml)	1210.8 ± 180.9	2408.2 ± 355.0	5445.2 ± 1016.3	6935.3 ± 862.0	8972.3 ± 1225.8	13146.0 ± 1185.2
Vz_obs (ml/kg)	402.7 ± 67.0	461.8 ± 84.1	489.6 ± 108.3	538.4 ± 86.4	600.1 ± 95.0	487.5 ± 58.8
Cl_obs (ml/h/kg)	163.1 ± 26.0	165.8 ± 22.9	149.5 ± 27.1	174.0 ± 21.7	179.7 ± 26.3	152.1 ± 13.7
Dose Ratio 1: 2: 4: 6: 8: 10						
AUC last Ratio 1: 2: 4: 6: 7: 11						
Continuous dose						
Dosage n (%) Mean ± SD	0.4 mg/kg +0.15 mg/kg/h for 24 h (4 mg/kg) (N = 6)		0.4 mg/kg +0.3 mg/kg/h for 24 h (7.6 mg/kg) (N = 6)		0.4 mg/kg +0.45 mg/kg/h for 24 h (11.2 mg/kg) (N = 6)	
HL_Lambda_z (h)	2.5 ± 0.2		2.5 ± 0.2		2.4 ± 0.3	
Tmax (h)	0.083 ± 0		0.083 ± 0		0.083 ± 0	
Cmax (ng/ml)	5472.1 ± 664.3		5398.9 ± 693.8		4400.0 ± 507.4	
AUC last (h*ng/ml)	29884.5 ± 5119.2		61627.6 ± 5497.9		64940.3 ± 10858.4	
Vz_obs (ml/kg)	489.3 ± 83.3		453.4 ± 60.2		615.2 ± 153.2	
Cl_obs (ml/h/kg)	136.7 ± 22.6		123.9 ± 10.6		176.7 ± 32.1	
Dose Ratio:1:1.9:2.8						
AUC last Ratio:1:2.1:2.2						

Abbreviations: AUC last, area under the concentration–time curve (AUC) from time zero to the last quantifiable concentration; Cl_obs, total body clearance of observation; Cmax, maximal drug concentration in plasma; HL_Lambda_z, half-life for the elimination phase; Tmax, time to maximum observed concentration; Vz_obs, apparent distribution volume of observation.

the exposure of hirudin in serum gradually increased and the trend of EH plasma concentration was similar to that of hirudin, showing a linear relationship. The concentrations of hirudin and EH in each dose group were below the lower limit of quantification after 28 and 36 h of continuous administration, respectively. The 0.45 mg/kg/h group showed no significant accumulation after continuous administration, and the accumulation ratio AR_Cmax was less than 1.25. At the doses of 0.15 and 0.30 mg/kg/h, the cumulative excretion rates of EH in urine from the start of administration to 48 h after the end of administration were 4.6% and 9.9%, while the cumulative excretion rates of hirudin were 0.6% and 1.2%, respectively (Table 6). The total cumulative excretion rates of EH and hirudin were 5.2% and 11.0%, respectively, suggesting that some other metabolites were produced from EH.

4 | DISCUSSION

4.1 | Safety

This trial was a first-in-human trial conducted in healthy volunteers to explore the safety, tolerability, and pharmacokinetics of EH. The action mechanism and structure determined showed that EH is highly safe and has low bleeding characteristics, as already proven in

preclinical studies. In this study, the safety and tolerance of EH was reconfirmed in healthy subjects within a single dose of 0.2–2 mg/kg and a continuous 24 h intravenous dose of between 0.15 and 0.45 mg/kg/h. Although the levels of hirudin *in vivo* increased with the increase in EH dose, these levels were still very low resulting in the absence of significant bleeding events. Adverse events have mostly been reported in clinical studies or applications of hirudin, including those related to bleeding and abnormal coagulation tests, such as hypohemoglobin and reticulocyte count increase[23-25]; however, these events were immediately controlled following symptomatic treatment or without treatment.

Activated partial thromboplastin time (APTT) is an important parameter for measuring both the therapeutic effect and the bleeding risk of hirudin, thus assisting in altering the therapeutic dose. Previous experiments have shown that hirudin significantly prolonged APTT, prothrombin time (PT), and thrombin time (TT), and the value of APTT was positively correlated with the plasma concentration of hirudin.[26,27] In this study, the prolongation of APTT was only observed in the 0.2 mg group (one subject) of the single-dose trial and in two subjects each from the continuous administration trial groups (0.30 and 0.45 mg/kg/h). The increase in APTT in the 0.2 mg group case occurred on the 8th day after administration when over 7 half-lives had elapsed; therefore, it was considered not to be related to the study drug. The prolongation
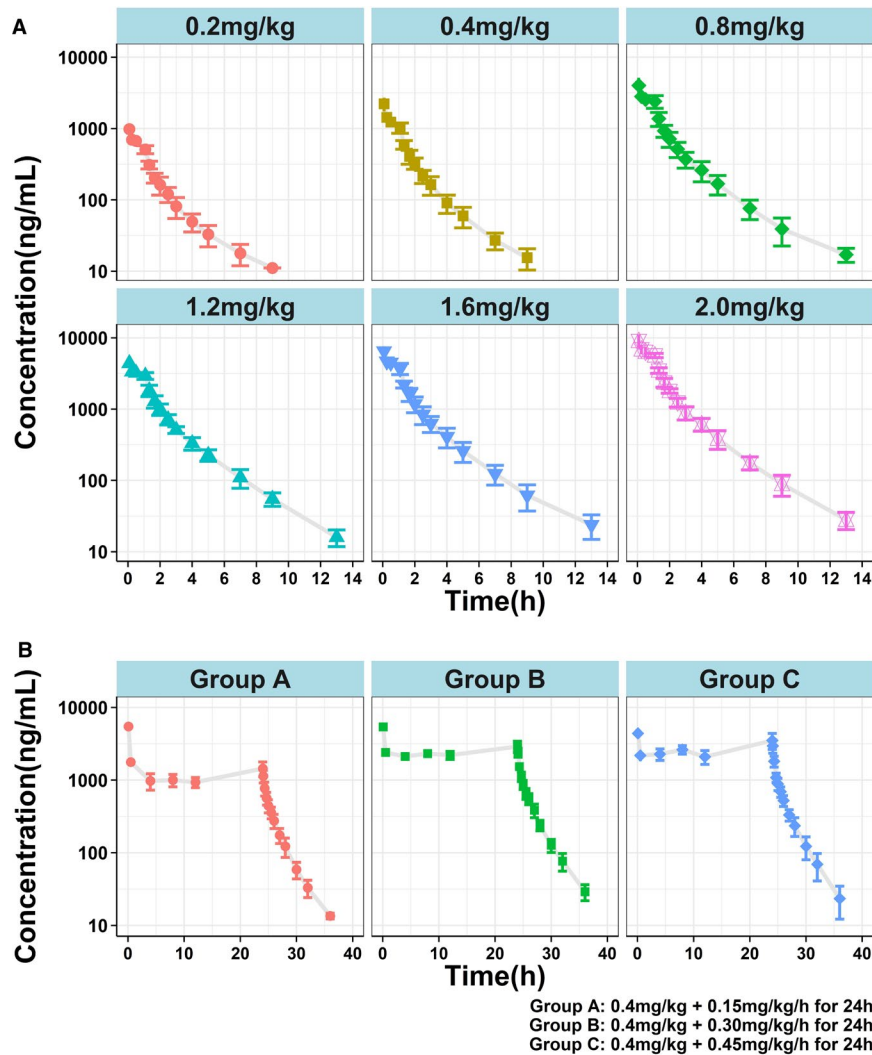
FIGURE 1 Plasma concentration- time profiles of EH following single-dose and continuous administration trials in healthy volunteers

Group A: 0.4mg/kg + 0.15mg/kg/h for 24h
Group B: 0.4mg/kg + 0.30mg/kg/h for 24h
Group C: 0.4mg/kg + 0.45mg/kg/h for 24h

TABLE 5 Ratio of hirudin to EH by dose after single-dose administration

Dosage n (%) Mean ± SD	0.2 mg/kg (N = 4)	0.4 mg/kg (N = 8)	0.8 mg/kg (N = 8)	1.2 mg/kg (N = 8)	1.6 mg/kg (N = 8)	2.0 mg/kg (N = 8)
EH AUC last (h*ng/ml)	1210.8 ± 180.9	2408.2 ± 355.0	5445.2 ± 1016.3	6935.3 ± 862.0	8972.3 ± 1225.8	13146.0 ± 1185.2
Hirudin AUC last (h*ng/ml)	44.2 ± 11.2	93.0 ± 19.4	347.4 ± 164.4	240.2 ± 50.5	220.7 ± 57.4	329.6 ± 106.8
Ratio %	3.7 ± 0.9	4.0 ± 1.2	6.3 ± 2.2	3.5 ± 0.9	2.4 ± 0.5	2.5 ± 0.8

of APTT in 4 subjects form the continuous administration trial occurred on the day of administration, which could be related to the action of EH. However, the symptoms were all mild and the subjects recovered on the same day or the next day without any treatment. The slight prolongation in APTT suggested that a small amount of hirudin was released with an increase in EH loading, but the hirudin level in healthy subjects remained at low levels after continuous administration, indicating that EH was generally safe in healthy adults.

Furthermore, an increase in fibrin D-dimer and a decrease in fibrinogen were observed in several subjects during the study. Numerous studies[28-30] have indicated that D-dimer levels are typically elevated in acute VTE. However, D-dimer levels may also increase in a variety of non-thrombotic disorders such as lipemia, hyperbilirubinemia, and hemolysis. In hospitalized and other commonly affected acutely ill patients, D-dimer testing has less utility because of the high frequency of false-positive results. Therefore, the significance of D-dimer increase in this study needs to be further investigated in future clinical studies. The decrease in fibrinogen in this study was difficult to explain using the mechanisms associated with a reduction in fibrinogen concentration, viz. hemodilution, consumption, and degradation. Nevertheless, a decrease in fibrinogen

TABLE 6 Cumulative excretion rate of EH and hirudin in urine (%)

Dosage n (%)	0.4 mg/kg + 0.15 mg/kg/h for 24 h (4 mg/kg) (N = 6)		0.4 mg/kg + 0.3 mg/kg/h for 24 h (7.6 mg/kg) (N = 6)	
	EH	Hirudin	EH	Hirudin
0–4 h after the start of administration	1.609 ± 1.281	0.197 ± 0.225	1.422 ± 0.720	0.169 ± 0.132
4–8 h after the start of administration	0.504 ± 0.212	0.029 ± 0.010	1.909 ± 0.677	0.112 ± 0.037
8–12 h after the start of administration	0.688 ± 0.509	0.033 ± 0.008	1.922 ± 1.392	0.109 ± 0.058
12–16 h after the start of administration	0.607 ± 0.368	0.067 ± 0.074	1.314 ± 0.653	0.108 ± 0.125
16–20 h after the start of administration	0.476 ± 0.371	0.128 ± 0.091	2.549 ± 1.061	0.438 ± 0.149
20–24 h after the start of administration	0.692 ± 0.688	0.185 ± 0.172	2.910 ± 3.212	0.495 ± 0.253
0–4 h after the end of administration	0.158 ± 0.263	0.030 ± 0.040	0.384 ± 0.239	0.045 ± 0.054
4–8 h after the end of administration	0.007 ± 0.0005	ND	0.025 ± 0.020	0.015 ± 0
8–12 h after the end of administration	0.007 ± 0.003	ND	0.010 ± 0.014	ND
12–16 h after the end of administration	0.003 ± 0	ND	0.003 ± 0.001	ND
16–20 h after the end of administration	ND	ND	ND	ND
20–24 h after the end of administration	ND	ND	ND	ND
24–32 h after the end of administration	ND	ND	ND	ND
32–40 h after the end of administration	ND	ND	ND	ND
40–48 h after the end of administration	ND	ND	ND	ND
Mean cumulative excretion rate (%)	4.6	0.6	9.9	1.2
Mean cumulative excretion rate of EH and hirudin (%)	5.2	-	11.0	-

levels can be demonstrated by an evaluation of D-dimer and other fibrin degradation products.[31-33]

4.2 | Pharmacokinetics

EH showed predictable pharmacokinetics after intravenous administration, with exposures increasing proportionally with dose. The pharmacokinetic parameters of EH in healthy volunteers were consistent with those reported in preclinical studies of monkeys and rats.

The concentration of EH in serum reached its peak within 5 min after the end of administration, with exposures (AUC and Cmax) increasing proportionally with doses between 0.2 and 2.0 mg/kg in the single-dose and 0.15–0.45 mg/kg/h (24 h) in the continuous dose administration trials. After intravenous administration, EH was mainly distributed in the extracellular fluid with an apparent volume of distribution of between 402.7 and 615.2 ml/kg, which was independent of dosage.

Preclinical animal studies have suggested that renal excretion is the major excretion route of EH, with urine excretion accounting for 65.9% of the total dose, determined by isotope labelling of [131]I. However, in the current clinical study, the cumulative excretion rate in urine of prototype drugs plus hirudin was about 10%, which indicated that EH produced some metabolites other than hirudin. Undeniably, with the exception of EH and hirudin, the two major measurable metabolites in human urine after intravenous administration of EH were found to be truncated at the C-terminus of EH

and hirudin. Owing to this, a successive reduction in amino acids at the C-terminus was reported, suggesting that EH was metabolized to produce hirudin and other metabolites, and that hirudin was metabolized to corresponding metabolites by the kidney.[17] These results suggested that the metabolic pathway of EH in vivo was similar to that of hirudin.[18] Moreover, the pharmacokinetic parameters and excretion of EH were also similar to those of hirudin.[20]

The pharmacokinetic parameters of hirudin and recombinant hirudin have been characterized in healthy volunteers.[34] Native hirudin and recombinant hirudin undergo little, if any, hepatic metabolism. In hirudin studies, more than 70% of the drug was excreted unchanged in the urine within the first hour after intravenous administration, and 95% was eliminated after 5 h.[25] Following single-dose intravenous administration in healthy volunteers, both native hirudin and recombinant hirudin had rapid distribution phases. The half-life ($T_{1/2}$), maximum plasma concentration (Cmax), and volume of distribution (Vd) of the drugs ranged from 0.15 to 1.24 h, 0.6 to 1.0 mg/L, and 8.9 to 17.2 L, respectively.[34-36] These results suggested that the pharmacokinetic parameters of EH were similar to those of hirudin.

The ratio of the area under the curve of hirudin to EH was 4.0% ± 1.2%, indicating that a small amount of EH was cleaved into hirudin, resulting in the high safety profile and low bleeding potential of EH. In healthy organisms, the observed levels of activated FXa and FXIa were low [37] and EH mainly existed in its intact form (very low levels of the active metabolite). However, the relationship between the activity level of hirudin and the in vivo hypercoagulable state of EH is not clear, and further studies are needed to establish the dose–effect relationship of EH in patients.

Interestingly, no hirudin was detected in the plasma samples of the 0.30 mg/kg/h group of the continuous dose administration trial, while there was no significant difference in the concentration of EH between plasma and serum, which indicated that serum hirudin may have come from the process of blood coagulation *in vitro* through activated coagulation factors.

Considering the small sample size in this study, the clinical therapeutic effect of EH should be confirmed by conducting a phase II study.

4.3 | Limitations

There were a few limitations to the conclusions drawn from this study. Because the subjects were healthy, the pharmacokinetic data collected represented the best case and did not include variability due to patient covariates. Since the study was performed in a small number of subjects, conclusions related to safety can only be made for common adverse events, but not rare adverse events.

5 | CONCLUSIONS

The results of this study showed that EH had good tolerance and safety profiles in Chinese healthy subjects, but the safety and pharmacodynamics of this drug need to be further studied in patients with thrombosis. No dose-limiting toxicity, deaths, serious adverse events or serious adverse reactions, and adverse events or adverse reactions leading to withdrawal occurred. In addition, most adverse reactions were controlled and resolved without intervention. Overall, the tolerance and PK characteristics of EH support that phase II clinical trials of this drug can be performed using the recommended doses of 4–11.2 mg/kg, with the recommended administration procedure: intravenous bolus injection of 0.4 mg/kg, followed by a maintenance infusion dose of 0.15–0.45 mg/kg/h.

6 | AUTHOR CONTRIBUTORS

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this study.

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