Int J Clin Exp Med 2017;10(5):8018-8024
www.ijcem.com /ISSN:1940-5901/IJCEM0048205

Original Article

Pharmacokinetics, pharmacodynamics and tolerability of single dose of recombinant glucagon-like peptide-1 receptor agonist in healthy Chinese subjects

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Received June 13, 2016; Accepted March 18, 2017; Epub May 15, 2017; Published May 30, 2017

Abstract: The objectives of the present study were to assess the pharmacokinetics (PK), pharmacodynamics (PD), tolerability and safety of lyophilized recombinant glucagon-like peptide-1 receptor agonist (rE-4) in healthy Chinese subjects. This randomized, double-blind, placebo-controlled, single-dose escalation study was conducted in 46 healthy Chinese subjects who received rE-4 (2.5 μg, 5 μg, 10 μg, 12.5 μg, 15 μg, or 17.5 μg) or placebo. The PK parameters of rE-4 were derived using non-compartmental models with WinNonLin software. Blood samples were also collected to assess the effects on blood glucose. The major PK parameters were as follows: C_{max} 119.96±25.09 to 514.07±41.57 pg/ml; T_{max} approximately 1.0 h; $t_{1/2}$ approximately 1.7 h; $AUC_{0\text{-}tn}$ 250.88±32.34 to 1739.16±137.94 h*pg/ml. The glucose level decreased in different degrees in all rE-4 dose groups. A total of 13 adverse events were reported in 11 of 34 subjects following rE-4 administration. The data suggest an approximately dose-proportional increase in $AUC_{0\text{-}tn}$ values, whereas C_{max} values increased less than proportionally over the 2.5 μg to 15 μg dose range, while the other major PK parameters were similar. Blood glucose monitoring showed a dose-dependent hypoglycemic effect of rE-4 which was well-tolerated in the studied dose range.

Keywords: Glucagon-like peptide-1 receptor agonist, pharmacokinetics, pharmacodynamics, tolerability, healthy Chinese subjects

Introduction

Type 2 diabetes mellitus (T2DM) is associated with progressive β cell dysfunction together with insulin resistance. A new perspective in the clinical treatment of diabetesis the use of incretin hormones. Glucagon-like peptide-1 (GLP-1) is a brain-gut insulintropicpeptide that plays an important role in the regulation of glucosehomeostasis [1, 2]. GLP-1 is produced by L cells present in the mucosa of the distal small intestine and the colon; its secretion is stimulated by the ingestion of carbohydrates or other nutrients [3]. Native GLP-1 has a short half-life of a few minutes, being rapidly degraded by dipeptidyl peptidase-IV (DPP-IV).

The agent used in this study, rE-4, was produced as a sterile lyophilized preparation by recombinant DNA technology and expressed in *Escherichia coli* and has the same amino acid sequence asexenatide (Byetta®) (**Figure 1**) [4], with the exception that rE-4 is without C-terminal amidation. Exenatide is a synthetic peptide that was originally identified in the lizard *Helodermasuspectum*, and was approved in 2005 as the first in a class of new agents for treating type 2 diabetes. In patients with type 2 diabetes, exenatide reduces post-prandial and fasting plasma glucose concentrations [6]. It is a potent and long-acting GLP-1 receptor agonist, which shows 53% sequence similarity to mammalianglucagon-likepeptide-1 (GLP-1) [6, 7]. Exenatide has been shown to share the glucose-regulating properties of GLP-1, including glucose-dependent enhancement of insulin secretion [8-11] and suppression of inappropriately high glucagon secretion [10], slowing of gastric emptying [10, 12], which may be paradoxically accelerated in people with diabetes

Exenatide

H-His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser-NH_2.

rE-4

H-His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser.

Figure 1. Amino acid sequence of exenatide and rE-4.

[13], and appetite suppression [14]. However, exenatide differs substantially from GLP-1 in terms of pharmacokinetics. The substitution of the Gly2 amino acid for an Ala2 at the inactivation site makes exenatide more resistant to dipeptidyl peptidase-4, which accounts for its much longer half-life and facilitates its therapeutic use [15, 16].

In vitro and in vivo studies have shown that rE-4 shares similar PD characteristics with exenatide, compared with synthetic exenatide, rE-4 production is more environmentally friendly and cost-effective. This is the first study of the pharmacological features of rE-4 in humans, which is of considerable interest in determining the potential of this agent for the treatment of T2DM. The purpose of this dose-escalation study was to investigate the pharmacokinetics, safety, and tolerability of a single-dose of rE-4, as well as to determine the effects on blood glucose level in healthy adult individuals.

Materials and methods

The study was conducted in accordance with the Declaration of Helsinki [World Medical Association], Good Clinical Practice (GCP) guidelines and the laws and regulations of the People's Republic of China. The study protocol and informed consent forms were approved by the Independent Ethics Committee and the Institutional Review Board of Peking University First Hospital and the China Food and Drug Administration (CFDA). Prior to the beginning of the study, all subjects provided written informed consent.

Volunteers

Healthy male or female Chinese individuals aged 18-45 years, with a body mass index (BMI) of 19-25 kg/m^2 and weight over 50 kg, were enrolled. Female subjects were not pregnant or did not have childbearing potential. Subjects were ineligible if any abnormalities in the clinical laboratory were identified at the screening visit or their fasting blood glucose levels were higher than 6.1 mmol/L or lower than 2.8 mmol/L, Subjects who had systolic pressure lower than 90 mmHg or higher than 140 mmHg, or diastolic pressure higher than 90 mmHg were also excluded. Other exclusion criteria were known hypersensitivity to the study drug and evidence-indicated drug abuse and so on.

Study design

This randomized, double-blind, placebo-controlled, single-dose escalation study was conducted at a single site. A total of 46 subjects (24 males and 22 females) were divided randomly into the rE-4 groups (2.5 μg, 5 μg, 10 μg, 12.5 μg, 15 μg, or 17.5 μg).

Subjects entered the study unit the day before drug administration. A pre-study investigation was conducted in which two subjects received rE-4 (2.5 μg) via subcutaneous injection. Based on the results, rE-4 was administered in the dose range 2.5 μg-17.5 μg in the formal study. The rE-4 groups comprised 6 subjects per group (except the 17.5 μg dose group, which comprised 4 subjects) and the placebo group comprised 12 subjects. Blood samples were collected from subjects in the rE-4 (2.5 μg-15 μg) dose groups for pharmacokinetic analysis.

Safety and tolerability assessment

All subjects were continuously observed in the study unit. The severity was classified according to the Medical Dictionary for Regular Activities criteria. Subjects responded to non-leading, non-specific questions and clinical observation and assessment. Safety evaluations including physical examinations, vital

Table 1. Baseline demographic characteristics of subjects enrolled in the study

	rE-4 dose						
	Placebo (n=12)	2.5 μg (n=6)	5 μg (n=6)	10 μg (n=6)	12.5 μg (n=6)	15 μg (n=6)	17.5 μg (n=4)
Sex (male/female; n)	4/8	4/2	4/2	4/2	3/3	4/2	3/1
Age (range; years)	31.2±2.7 (28, 37)	32.7±2.3 (30, 36)	32.3±2.5 (30, 37)	30.0±2.1 (28, 33)	31.7±3.9 (28, 37)	32.2±2.9 (29, 37)	32.5±2.6 (30, 36)
Height (cm)	161.1±7.5	163.3±7.8	166.2±6.6	165.3±11.3	165.2±3.8	164.8±9.1	161.8±2.9
Weight (kg)	61.9±6.6	61.8±5.4	65.8±6.7	64.5±8.5	63.3±4.4	60.2±8.5	60.4±5.5

Values are shown as mean ± SD, except for age, which is shown as mean (range). BMI: body mass index.

Table 2. Summary of adverse events reported during this study

Dose group	No. of Subject	Event Terminology	Severity	Event Relationship
2.5 μg	2	Hypoglycemia	Mild	Likely
5 μg	9	Blood glucose level decreased[a]	Mild	Likely
	15	Blood glucose level decreased	Mild	Likely
		WBC[b] decreased	Mild	Likely
10 μg	21	Nausea	Mild	Probable
12.5 μg	25	Blood glucose level decreased	Mild	Likely
	31	Blood glucose level decreased	Mild	Likely
	32	Nausea	Mild	Probable
15 μg	39	Blood glucose level decreased	Moderate	Likely
		WBC & NE%[c] increased	Mild	Probable
17.5 μg	41	Blood glucose level increased	Mild	Suspicious
	43	Nausea	Mild	Probable
	45	Nausea	Mild	Probable

Note: a. Blood glucose level decreased means the glucose level decreased within normal limits. b. WBC = white blood cell. c. NE = neutrophilicgranulocyte.

signs, 12-lead electrocardiogram (ECG), clinical chemistry, urinalysis, blood glucose monitoring, and chest X-rays were conducted at scheduled intervals.

Sample analysis

For the PK analysis, blood samples were collected at pre-dose, 15 min, 30 min, 45 min, 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 8 h, and 12 h post-dose according to the results of the pre-study. Blood samples were collected into tubes containing ammonium heparinate and centrifuged immediately at 4°C to obtain plasma samples. These samples were then transferred to polypropylene vials and stored -70°C until analysis.

For the PD analysis, blood samples were collected at pre-dose, 15 min, 30 min, 45 min, 1 h, 1.5 h, 2 h, 3 h, and 4 h post-dose. Blood samples were stored in vacuum tubes containing sodium fluoride. These samples were sent to clinical laboratory of Peking University First Hospital and assessed using the glucose oxidase method. Real-time monitoring of blood glucose levels was performed using a fast blood glucose meter (ACCU-CHEK®).

Method validation

In this study, the PK samples were analyzed for rE-4 using fluorescent immunoassay kits (Phoenix Pharmaceuticals, USA). This method was linear in the range of 12.5-400 pg·mL^{-1}, and the standard sample were diluted 10, 20 and 40 times (the accuracy was within ±4.66, CV ≤ 7.71%). The average recovery rate was 101.6% (CV < 13.5%) and the quantitative limit of this methodwas 12.5 pg·mL^{-1} (CV 4.63%). Therefore, the accuracy, precision and sensitivity of this method were confirmed to be suitable for the determination of the effects of rE-4.

Pharmacokinetic and pharmacodynamics parameters

The PK parameters assessed were: area under the plasma concentration-time curve ($AUC_{0\text{-}tn}$, $AUC_{0\text{-}\infty}$), maximum plasma concentration (C_{max}), terminal half-life ($t_{1/2}$), clearance (CL), apparent volume of distribution (V), and mean residence time (MRT).

The PD parameters were blood glucose values. The blood glucose-time curve and mean blood glucose-time curve of each subject were plotted in the different dose groups, and compared with the placebo group , to explore the pharmacological effects of rE-4 on blood glucose.

Statistical analyses

The PK parameters of rE-4 were analyzed using non-compartmental methods with WinNonlin Professional Version 6.0 (Phoenix) (Pharsight Corporation, Mountain View, CA, USA). C_{max} and T_{max} were identified from a measured sample. The terminal phase rate constant (λz) was determined using linear regression of the terminal linear portion of the semi-logarithmic plasma concentration-time data. The plasma terminal half-life ($t_{1/2}$) was calculated as 0.693/λz. The area under the plasma concentration-time curve to the last measurable time-point ($AUC_{0\text{-}t}$) was determined by the linear trapezoidal rule. All pharmacokinetic parameters were summarized using descriptive statistics.

Results

Demographic characteristics

The baseline demographics for the study subjects are shown in **Table 1**. There were no early discontinuations during the study.

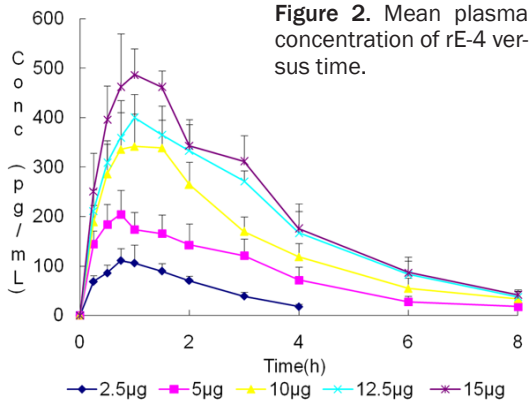

Figure 2. Mean plasma concentration of rE-4 versus time.

Safety and tolerability

There were no serious adverse events (SAEs) or deaths reported during the study and all subjects were in good compliance. The most common adverse events (AEs) were decreased blood glucose levels (5 cases; 1 moderate and 4 mild) and nausea (4 cases) (see **Table 2**). The incidence of AEs in each group were 16.7%, 33.3%, 16.7%, 50%, 16.7%, and 50% (2.5 μg-17.5 μg dose groups, respectively), and 0% (placebo group).

Pharmacokinetics

The mean plasma concentration-time profiles of rE-4 are shown in **Figure 2**. The plasma concentrations of rE-4 were generally measurable at all-time points post-dose, with the exception of the 2.5 μg dose group, for which the levels were undetectable from at 6 h post-dose. Individual C_{max} values ranged from 119.96 pg/mL (2.5 μg dose group) to 514.07 pg/mL (15 μg dose group). The pharmacokinetic parameters values estimated by non-compartmental procedures are listed in **Table 3**. Plasma concentrations of rE-4 declined with an approximate mean apparent terminal half-life of 1.09-1.94 h following administration.

Preliminary pharmacodynamics

All subjects had breakfast 30 minutes after receiving rE-4 or placebo. The blood glucose in all rE-4 groups decreased within the first 45 min, followed increased in different degree after breakfast. However, rise or fall was both in the normal range.

After a single dose of subcutaneous injection of 2.5 μg~15 μg rE-4 or placebo in healthy Chinese subjects, Blood glucose level in most of rE-4 received groups decreased at the first 45 minutes then slowly increased after breakfast. The placebo group presented no change of blood glucose level within first 45 min, increased after breakfast until 1 hour, then decreased. The change of mean blood glucose of each rE-4 received group abstract placebo group at different time point versus time were shown as **Figure 3**, compare with placebo group, blood glucose level of rE-4 group decreased remarkably and can maintain about 150~240 minutes, then increased slowly and mildly higher than placebo group.

Discussion

This is the first study of the pharmacokinetic and pharmacodynamic properties of rE-4 in humans. The pre-study was conducted with a minimum dose of 2.5 μg administered to 2 subjects to observe the safety and adjust the blood collection points for pharmacokinetic analysis. The highest dose of rE-4 in humans was calculated to be 38 μg based on the completely safe dose in Wistar rats. The highest tolerated dose of exenatide is approximately 18 mg, with the incidence of nausea, vomiting and other adverse events increasing with the dose. Finally, 17.5 mg was selected as the highest dose of rE-4 administered in this study.

No severe adverse events were observed in this study. There was no clear relationship between the severity of AEs and the dose of rE-4, although the incidence, especially in the case of nausea, increased with the dose. Therefore, the recommended dose for use in Phase II clinical studies should not exceed 17.5 μg.

In addition to assessing tolerability and safety, the present study represents the first evaluation of the pharmacokinetics of rE-4 in humans. As in previous studies, the plasma concentration of rE-4 was determined by ELISA. To ensure the accuracy and reliability of the results, a new standard curve was established for each batch of samples and three quality control samples (low, middle and high concentration) were also included in each assay.

Linear analysis with WinNonLin 6.0 (Phoenix) showed that there was an approximately dose-proportional increase in AUC values following

Table 3. Summary of mean pharmacokinetic parameters of rE-4 after single dose of 2.5 μg, 5 μg, 10 μg, 12.5 μg, and 15 μg

Parameters	rE-4 dose				
	2.5 μg n=4	5 μg n=6	10 μg n=6	12.5 μg n=6	15 μg n=6
$t_{1/2}$ (h)	1.09±0.15	1.70±0.32	1.90±0.64	1.94±0.15	1.83±0.36
C_{max} (pg/mL)	119.96±25.09	217.46±37.22	388.15±34.16	426.05±34.83	514.07±41.57
T_{max} (h)	0.81±0.13	0.67±0.13	1.13±0.44	1.29±0.46	1.08±0.34
AUC_{0-tn} (h*pg/mL)	250.88±32.34	683.85±137.08	1177.37±119.30	1528.96±179.37	1739.16±137.94
$AUC_{0-\infty}$ (h*pg/mL)	279.32±28.88	726.43±137.57	1273.47±168.75	1634.02±202.18	1852.52±146.12
V (L)	14.30±3.03	17.41±4.56	21.08±4.87	21.78±3.82	21.40±4.50
Cl (L/h)	9.03±0.98	7.04±1.03	7.99±1.22	7.74±0.93	8.14±0.61
MRT_{0-tn} (h)	1.60±0.09	2.46±0.32	2.49±0.27	2.70±0.17	2.62±0.22

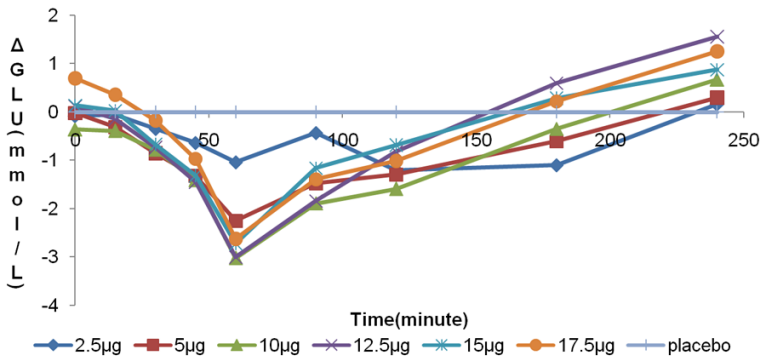

Figure 3. ΔMean blood glucose versus time profiles in different treatment groups (each rE-4 received group: n=6; placebo group: n=12). Note: 1. each group had breakfast 30 minutes after receiving rE-4 or placebo. 2. ΔGLU = Mean blood glucose of each dose group at different time point- Mean blood glucose of placebo group at different time point.

administration of a single dose of 2.5 μg-15 μg rE-4 (90% CI: 63%-80%), whereas C_{max} increased disproportionately (90% CI: 93%-123%), indicating a nonlinear pharmacokinetic profile. There were no significantly differences between the groups in the other main pharmacokinetic parameters. Interpretation of these data is limited by the small number of samples and further studies including greater number of subject are required to confirm our findings.

The main pharmacokinetic parameters reported after subcutaneous injection of exenatide (5 μg) in healthy Chinese subjects were reported to be: C_{max} 135 pg/ml, AUC_{0-tn} 381 h*pg/mL, $t_{1/2}$ 1.15 h, V 22.3 L, and CL 13.1 L/h; while, when the dose was increased to 10 μg the parameters were as follows: C_{max} 315 pg/ml, AUC_{0-tn} 837 h*pg/mL, $t_{1/2}$ 1.25 h, V 21.7 L, CL 12.0 L/h [17]. After a single dose of rE-4 (2.5 μg-15 μg) administered by subcutaneous injection, pharmacokinetic evaluations revealed that rE-4 was absorbed rapidly since the T_{max} was 0.6-1.3 h, and declined with a terminal half-life of 1.09-1.94 h. The apparent volume of distribution was approximately 14-21 L, and apparent clearance was 7.0-9.0 L/h. The pharmacokinetic properties of rE-4 observed in this study showed some consistency with those of exenatide in that the $t_{1/2}$, V and CL can compare well with those of exenatide, although rE-4 showed higher system exposure parameters, such as C_{max} and AUC_{0-tn}.

In the present study, all rE-4 groups were associated with reductions in glucose levels compared with those observed in the placebo group. Glucose monitoring showed that the levels decreased to different degrees in all rE-4 dose groups after administration and before breakfast, while the blood glucose levels in the placebo group remained unchanged. Postprandially, the blood glucose levels in the placebo group increased significantly, and then decreased, while, this increase was less marked in the rE-4-treated groups, showing a dose-dependent effect that can be maintained for 150-240 min. The blood glucose levels in the higher rE-4 dose groups decreased remarkably, with the effects showing an increasing trend with decreased rE-4 concentrations. It can be speculated that this phenomenon is caused by the actions of hormonal insulin antagonists secreted in response to hypoglycemia [18].

Acknowledgements

This study was financially supported by CSPC Zhongqi Pharmaceutical Technology (Shijiazhuang) Co., Ltd. The author thanks the investigators who were involved in this clinical trial for their assistance in the study: Drs. Dongmei Zhang, Xiaoyan Sheng, Zining Wang, Jingzi Li and Shuohan Tian.

Disclosure of conflict of interest

None.

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