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ORIGINAL RESEARCH

First-in-Human Safety, Tolerability, and Pharmacokinetics of Single-Dose Kukoamine B Mesylate in Healthy Subjects: A Randomized, Double-Blind, Placebo-Controlled Phase I Study

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ABSTRACT

Introduction: Kukoamine B mesylate (KB) is a mesylate chrysamine B targeting lipopolysaccharides and CpG DNA, two potential treatment targets in sepsis.

Methods: This first-in-human, randomized, double-blind, placebo-controlled, phase I study was conducted from July 2014 to May 2015 to explore the safety, tolerability, and pharmacokinetics of KB in healthy subjects. This study consisted of a pre-phase (four participants; KB at 0.005 mg/kg) and a dose escalation phase (eight participants/dose group, randomized 6:2 to KB or placebo; KB at 0.02, 0.04, 0.08, 0.12, 0.24, and 0.48 mg/kg). The primary endpoint was safety.

Results: Fifty-two participants were enrolled, including four in the pre-phase and 48 in the dose escalation phase. Among the 40 participants who received KB, 12 (30.0%) experienced adverse events (AEs), while two (16.7%) experienced AEs among 12 participants who received the placebo. The most common AEs in the KB group were headache (5.0%), influenza (5.0%) and positive white blood cell in urine (5.0%). After the administration of KB, the mean plasma elimination half was around 1.61–4.24 h. The relationship between the KB plasma exposure of KB and the administered dose was not linear. The percentage of cumulative urinary excretion of KB was similar among the different dose groups (21.7–35.2%) and the urinary excretion of KB decreased significantly about 8 h after administration.

Conclusions: Single-dose KB demonstrated favorable safety and tolerability in healthy subjects at the dose level of 0.005–0.48 mg/kg. KB exhibited a non-linear pharmacokinetic profile with a half-life of about 1.61–4.24 h, which mainly distributed in plasma.

Trial Registration: ClinicalTrials.gov identifier, NCT02219971.

Hongzhong Liu and Qian Zhao contributed equally to this work.

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Keywords: Kukoamine B; Phase I trial; Pharmacokinetic; Safety

Key Points

Why carry out this study?

This first-in-human, randomized, double-blind, placebo-controlled, phase I study was conducted to explore the safety, tolerability, and pharmacokinetics of KB in healthy subjects.

What was learned from this study?

Single-dose KB demonstrated favorable safety and tolerability in healthy subjects at the dose level of 0.005–0.48 mg/kg.

The findings of this study provide preliminary evidence for further clinical trials verifying the efficacy and safety of KB in sepsis.

INTRODUCTION

Sepsis is a life-threatening organ dysfunction caused by the dysregulated host response to infection [1, 2]. Approximately 750,000 cases of sepsis occur annually in the United States [3, 4]. In China, the incidence of sepsis is 20.6 cases per 100 intensive care unit (ICU) admissions, with a 30-day mortality of 36% [5]. Without timely treatment, sepsis may advance to septic shock, which is associated with high mortality (> 40%) [1, 2]. Therefore, improving the prognosis of patients with sepsis is one of the major public health challenges facing the world. The management of sepsis involves managing the underlying infection with antibiotics or antifungals, the inflammatory state (with corticosteroids), and supporting the vital functions (fluid therapy, vasoactive drugs, respiratory support, etc.) [1, 2], but there is a lack of specific drugs based on the pathophysiological mechanisms of sepsis.

Bacterial pathogen-associated molecular patterns (PAMPs) are recognized by pattern recognition receptors (PRRs) on the inflammatory cells of the innate immune system. This interaction mediates the activation of inflammatory cells and the release of cytokines, leading to a dysregulated host response [6]. The lipopolysaccharides (LPS) found on the membrane of Gram-negative bacteria are the most abundant and virulent PAMPs in Gram-negative bacteria [7]. Once the immune cells recognize LPS through the Toll-like receptor 4 (TLR4), they release pro-inflammatory cytokines; imbalances between pro- and anti-inflammatory cytokines can lead to sepsis [7]. Meanwhile, CpG DNA is a PAMP widely found in all bacteria [8]. Therefore, targeting LPS and CpG DNA may be a feasible direction for treating sepsis.

Kukoamine B mesylate (KB) is a mesylate chrysamine B prepared by a fully chemical synthesis method and can bind and neutralize LPS and CpG DNA, block the interaction of LPS and CpG DNA with their corresponding receptors (TLR4 and TLR9) on immune cells, inhibit immune cell activation, and reduce the production of inflammatory mediators such as tumor necrosis factor (TNF)-α and interleukin (IL)-6 [9–11]. In pharmacodynamics and preclinical animal experiments, KB had good antagonistic activity against pathogenic molecules of various major pathogenic bacteria, could significantly improve the survival rate of sepsis model animals, and presented a clear dose–effect relationship and a large safe dose range [9–11].

Therefore, given the activity of KB in preclinical studies, this first-in-human, randomized, double-blind, placebo-controlled phase I clinical trial explored the safety, tolerability, and pharmacokinetics of KB in healthy subjects. The results could provide evidence for further clinical trials of KB.

METHODS

Study Design and Participants

This trial was a randomized, double-blind, placebo-controlled, single-dose phase I study

conducted in Peking Union Medical College Hospital from July 2014 to May 2015. The study was performed in accordance with the International Conference on Harmonization Good Clinical Practice guidelines and ethical principles that have their origin in the Declaration of Helsinki of 1964 and its later amendments. This study was approved by the Ethics Review Board of Peking Union Medical College Hospital (approved no. 2014L01029). All participants provided written informed consent before enrollment. The trial was registered on ClinicalTrials.gov (NCT02219971).

The inclusion criteria were (1) healthy male or female (sex ratio of no more than 2/3 in each dose group), (2) 18–45 years of age, (3) body mass index (BMI) of 19–28 kg/m^2 and $\geq$ 50 kg for females and $\geq$ 60 kg for male, and (4) no parenting plan in the past 6 months and agreed to take effective contraceptive measures during the study, the women of childbearing age had negative blood pregnancy tests. The key exclusion criteria were (1) primary diseases in important organs, (2) mental or physical disabilities, (3) familial genetic diseases, (4) systolic blood pressure > 140 mmHg, diastolic blood pressure > 90 mmHg, or pulse > 100 bpm, (5) any clinically significant abnormality in physical examination, vital sign, electrocardiogram, or test results, (6) immunodeficiency diseases, (7) hepatitis B or C or treponema pallidum antibody, (8) alcohol and drug abuse, (9) those unable to abstain from smoke and alcohol during the study, (10) participated in any drug clinical trial within the past 3 months, (11) used any prescription drug within 4 weeks, used any non-prescription drug within 2 weeks, or took food that might affect drug-metabolizing enzymes within 2 weeks before enrollment, (12) blood donation or major blood loss (> 400 ml) in the last 3 months, (13) allergy or atopic allergic diseases, or known allergies to the study drug or drugs similar to the study drug, or (14) breastfeeding women, pregnant women, or those who could not take effective contraceptive measures.

Procedure

This study consisted of two parts: a pre-phase and a dose escalation phase. The pre-phase was open-labeled designed, with one dose group and four participants. A single-dose intravenous infusion of KB at 0.005 mg/kg was administered. Blood and urine samples were collected after administration, and the test results provided the basis for adjusting the blood/urine sampling time in the dose escalation phase. Firstly, one participant was selected, and the remaining three participants were enrolled after 2 days of observation to confirm that there were no safety concerns.

The dose escalation phase had a randomized, double-blind, placebo-controlled design. The maximum dose was firstly determined to be 0.12 mg/kg by fu-corrected intercept method based on the preclinical data of rat and dog. Considering the large safety dose range in animal models and variance of endotoxin level in humans, 0.24 and 0.48 mg/kg dose groups were added. Hence, dose groups of 0.02, 0.04, 0.08, 0.12, 0.24, and 0.48 mg/kg was defined. The eight participants in each group were randomly divided into the KB group and placebo group at a ratio of 6:2, given a single dose of KB or placebo, and observed for 7 days after administration. During the study, according to the safety and pharmacokinetic data of the subjects in the previous dose group, it was decided whether to escalate to the next dose group or terminate the dose escalation. The dose escalation termination criteria were (1) when $\geq$ 50% of the participants who received the study drug in a dose group had any grade 2 toxicity (excluding allergic reactions) or (2) $\geq$ 33% or more of the subjects had any grade 3 toxicity (excluding allergic reactions) (grading according to Common Terminology Criteria for Adverse Events 4.03 version).

Randomization was conducted by an independent third party using a computer-generated system, guaranteeing unbiased participant allocation. To maintain double-blind conditions, blinding envelopes containing group assignments were managed by an independent administrator. We appointed an independent third party responsible for overseeing the safety

aspects of the study. This party was tasked with making judgments about adverse events (AEs) and was not blinded to the subjects' grouping status. The key role of this independent evaluator was to decide on the continuation or termination of dose escalation, in accordance with the safety termination criteria set forth in the study protocol. This approach ensured continuous monitoring of participant safety and adherence to ethical standards throughout the course of the trial.

Endpoints

The primary endpoint was safety, which included AEs, clinical laboratory indicators, electrocardiogram, and vital signs. The severity of the AEs was determined as mild (did not affect daily life, no need for medical intervention), moderate (affected daily life, might require medical intervention), and severe (significantly affected daily life, required medical intervention). It was up to the investigators to determine whether the AEs were drug-related.

The secondary endpoints were the pharmacokinetic indicators: time-to-maximum (T_{max}), peak concentration (C_{max}), areas under the curve ($AUC_{0\text{-}t}$ and $AUC_{0\text{-}\infty}$), clearance (CL), distribution volume (Vd), half-life ($T_{1/2}$), prototype drug, and metabolites in urine and fecal samples. In the pre-phase, blood samples were collected before administration and after 20 and 40 min of infusion, immediately after the end of the infusion, 10, 20, 30, and 45 min, and 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, and 24 h after the end of infusion. Urine samples were collected before administration and 0–4, 4–8, 8–12, 12–24, and 24–48 h after the start of administration. In the dose escalation phase, the sampling points were adjusted according to the blood drug-concentration results in the pre-phase: before administration, 20 and 40 min after the start of the infusion, immediately after the end of infusion, and 30 min and 1, 2, 3, 4, 6, 8, 12, 16, and 24 h after the end of infusion. The sampling points of urine samples were adjusted as follows: before administration and 0–5, 5–9, 9–13, 13–25, and 25–49 h after the end of infusion. The content of KB and metabolites were determined quantitatively by liquid chromatography-mass spectrometry using the urine samples from participants received 0.12, 0.24, and 0.48 mg/kg KB. The participants in the 0.04, 0.08, 0.24, and 0.48 mg/kg dose groups collected fecal samples from the day before to 48 h after administration.

Statistical Analysis

In the context of this phase I clinical trial, the primary focus was on assessing the safety, tolerability, and pharmacokinetics of KB. Consequently, sample size calculations aimed at detecting specific treatment effects were not performed.

The pharmacokinetic parameters were computed using non-compartmental analyses and Phoenix WinNonlin 6.3 (Pharsight). The linear correlations between the pharmacokinetic parameters ($AUC_{0\text{-}inf}$, AUC_{last}, and C_{max}) of single administration and the doses were evaluated using Power Model in SAS 9.3 (SAS Institute Inc. Cary, NC, USA) using PROC MIXED (confidence level of 0.1). For AUC, the acceptable criteria for 90% confidence interval were 0.954–1.046 for AUC and 0.941–1.058 for Cmax. The continuous data that conformed to the normal distribution were presented as means ± standard deviation, and those that did not conform to the normal distribution were presented as median (range). The categorical data were presented as *n* (%). All results in this study were presented using descriptive statistics.

RESULTS

Baseline Characteristics of the Participants

Fifty-two healthy participants were included in this study, including four in the pre-phase and 48 in the dose escalation phase (Fig. 1). In the dose escalation phase, 36 subjects (six in each dose group) received KB, and 12 received the placebo. Supplementary Table S1 presents the baseline characteristics of the participants.

Safety and Tolerability

All participants completed the KB or placebo dosing according to the study protocol. In the total population, 14 participants (26.9%) experienced AEs. Among the participants who received KB, 12 (30.0%) experienced AEs. Among those who received the placebo, two (16.7%) experienced AEs (Table 1). The common AEs in the KB group were headache (5.0%), influenza (5.0%), and positive white blood cell in urine (5.0%).

All AEs were mild except for one case of serum creatine phosphokinase elevation, which was judged to be moderate. All AEs recovered spontaneously without any treatment. No serious AEs occurred. Except for two cases of influenza, which were judged by the investigators as possibly unrelated to the study drug, all other AEs were judged as possibly related to the study drug. No participants stopped the study because of AEs.

Pharmacokinetics

The detailed pharmacokinetic parameters are shown in Table 2. After the administration of 0.005, 0.02, 0.04, 0.08, 0.12, 0.24, and 0.48 mg/kg of KB, the plasma elimination half-lives were 1.61 ± 0.14, 2.25 ± 0.44, 2.78 ± 0.41, 3.97 ± 0.56, 3.20 ± 0.87, 4.24 ± 0.75, and 3.75 ± 0.34 h, respectively.

After a single intravenous infusion of 0.005, 0.02, 0.04, 0.08, 0.12, 0.24, and 0.48 mg/kg of KB in 40 healthy subjects, the plasma concentrations of KB increased gradually after the start of intravenous infusion and decreased rapidly after the end of infusion. The plasma drug–time curves of KB in each dose group were similar in shape (Fig. 2A). The increase of KB exposure that increased with the administered dose was slightly higher than that of the dose increase, and no dose-linear relationship was observed.

The evaluation of the linear relationship using the Power model showed that the plasma exposure of KB (AUC and C_{max}, as well as body weight-adjusted AUC and C_{max}) increased with increasing administered dose, but the proportion of increase was slightly higher than that of the dose increase, approximately 10–15% above the proportion, a strict dose-linear relationship was not shown, and neither the point estimate nor its 90% confidence interval fell within the acceptable range (Table 3). As shown in Fig. 3, after the intravenous infusion of KB at doses ranging from 0.005 to 0.48 mg/kg, the incidence of AEs was independent of dose and systemic exposure.

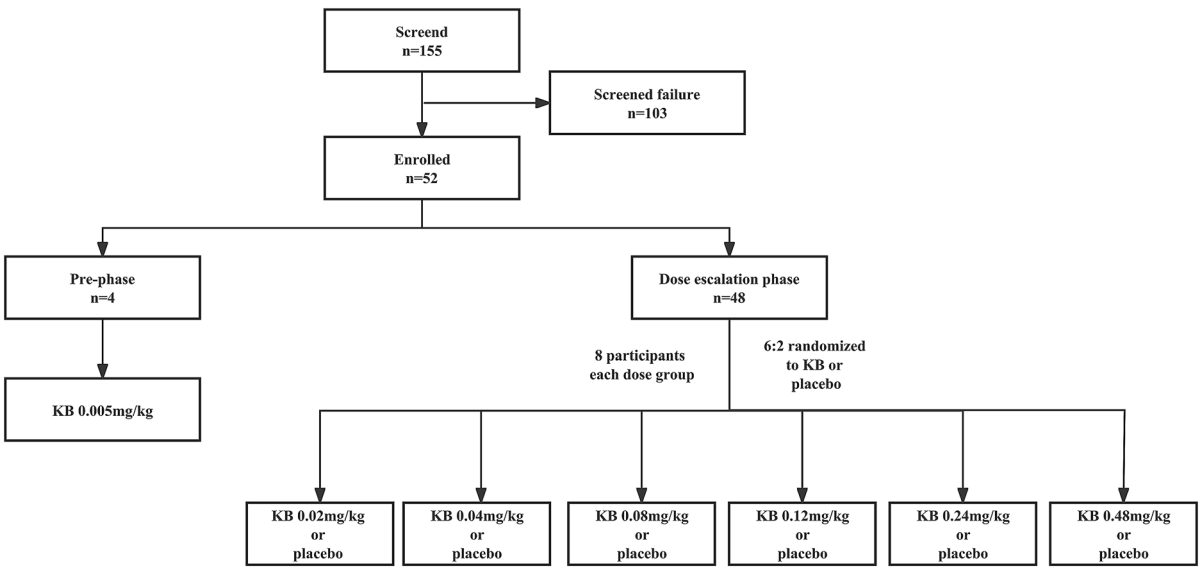

Fig. 1 Flow chart

Table 1 Adverse events

	0.005 mg/kg (*n* = 4)	0.02 mg/kg (*n* = 6)	0.04 mg/kg (*n* = 6)	0.08 mg/kg (*n* = 6)	0.12 mg/kg (*n* = 6)	0.24 mg/kg (*n* = 6)	0.48 mg/kg (*n* = 6)	KB (*n* = 40)	Placebo (*n* = 12)
Any AE	3 (75.0%)	2 (33.3%)	3 (50%)	0	0	2 (33.3%)	2 (33.3%)	12 (30.0%)	2 (16.7%)
SAE	0	0	0	0	0	0	0	0	0
Specific adverse event									
Elevated white blood cell count	0	0	0	0	0	0	1 (16.7%)	1 (2.5%)	0
Elevated ALT	0	0	0	0	0	0	1 (16.7%)	1 (2.5%)	0
Positive urinary white blood cell	0	0	2 (33.3%)	0	0	0	0	2 (5.0%)	0
Decreased hemoglobin	0	0	0	0	0	0	0	0	1 (8.3%)
Elevated CK	0	0	0	0	0	0	1 (16.7%)	1 (2.5%)	0
Headache	2 (50.0%)	0	0	0	0	0	0	2 (5.0%)	0
Muscle spasms	0	0	1 (16.7%)	0	0	0	0	1 (2.5%)	0
Influenza	0	1 (16.7%)	1 (16.7%)	0	0	0	0	2 (5.0%)	0
Chest discomfort	0	1 (16.7%)	0	0	0	0	0	1 (2.5%)	0
Irregular menstruation	0	0	0	0	0	1 (16.7%)	0	1 (2.5%)	0
Heavy menstrual flow	0	0	0	0	0	1 (16.7%)	0	1 (2.5%)	0
Nausea	0	1 (16.7%)	0	0	0	0	0	1 (2.5%)	0
Abdominal discomfort	1 (25.0%)	0	0	0	0	0	0	1 (2.5%)	0
Abdominal pain	1 (25.0%)	0	0	0	0	0	0	1 (2.5%)	0
Dry mouth	0	1 (16.7%)	0	0	0	0	0	1 (2.5%)	0
Palpitations	0	0	0	0	0	0	0	0	1 (8.3%)

AE adverse event, *ALT* alanine aminotransferase, *KB* kukoamine B mesylate, *CK* creatine kinase, *SAE* serious adverse event

Compared with the mean plasma drug concentrations of KB in the other dose groups, the drug concentrations of KB in whole blood were lower in the 0.24 and 0.48 mg/kg-dose groups, and the ratio of whole blood/plasma drug concentrations (B/P ratios) were 0.43 and 0.47, respectively, suggesting that KB was mainly distributed in the plasma. The whole blood $T_{1/2}$ of KB was 1.59 ± 0.29 and 1.78 ± 0.19 h, respectively, which were lower than the plasma $T_{1/2}$ (Supplementary Table S2). The whole blood concentration–time curves of KB at 0.24 and 0.48 mg/kg showed that the whole blood concentrations of KB gradually increased after the start of intravenous infusion and decreased rapidly after stopping the infusion. The whole blood concentration–time curves of KB were similar in shape for the two dose groups, with a gradual increase in whole blood exposure with increasing doses (Supplementary Figure S1).

The cumulative urinary excretion of KB gradually increased with the increased administered dose, and the percentage of cumulative urinary excretion of each dose group was similar, ranging from 21.7% to 35.2% (Fig. 2B). About 8 h after administration, the excretion of the prototype KB in the urine was significantly reduced. In quantitative analysis of KB and metabolite in urine samples, the average percentage of KB prototypes in urine samples during 49 h after KB administration in relation to the administrated KB was 39.65%, 33.97%, and 26.29% in 0.12, 0.24 and 0.48 mg/kg-dose group, respectively. The highest level of metabolite in urine sample was M7 (bis-methylated product), which accounted for 8.07%, 9.98%, and 9.88% of the dose administered in 0.12, 0.24, and 0.48 mg/kg-dose group, respectively, followed by M11 (bis-methylated, oxidatively deaminated carboxylic acid derivative, accounted for 4.01%, 5.75%, and 5.28%). The content of the other two forms of metabolites (M1, methylated *N*-desalkyl product; M4, cysteine binding product) were too little to be quantitatively analyzed (Supplementary Figure S2).

DISCUSSION

This first-in-human phase I trial aimed to explore the safety, tolerability, and pharmacokinetics of KB in healthy subjects. The results suggest that a single dose of KB demonstrated favorable safety and tolerability in healthy subjects at the dose level of 0.005–0.48 mg/kg. KB exhibited a non-linear pharmacokinetic profile with a half-life of about 1.61–4.24 h and was mainly distributed in plasma. The findings of this study provide preliminary evidence for further clinical trials verifying the efficacy and safety of KB in sepsis.

In this study, all AEs were mild except for one case of moderately elevated creatinine kinase. The most common AEs were influenza (5%), which was ruled to be possibly unrelated to KB, and headaches and positive white blood cell in urine, which were considered possibly related. A previous study in high-fat-fed rats showed that KB could decrease the levels of blood lipids and inflammatory cytokines and improve the markers of oxidative stress, but there were no data about common safety parameters like liver and kidney functions [12]. Another study revealed a decrease in inflammatory parameters in mice exposed to LPS without apparent toxicity [10]. Although these results are limited and preliminary, they suggest the safety of KB in humans.

Based on the present preliminary pharmacokinetic data, the exposure of KB in healthy subjects increased with the increase of the administered dose, but the proportion of the increase was slightly higher than that of the dose increase, which might be related to the characteristics of the study drug and factors such as the limited number of subjects. Still, such a non-linear relationship was also observed in a simulation study [13]. The whole blood $T_{1/2}$ of KB was lower than the plasma $T_{1/2}$, possibly because the lower limit of quantification (LLOQ) of the whole blood-based detection method was 100 times the LLOQ of the plasma-based detecting method, which made the whole blood sample at the end of the elimination phase less detectable than the plasma sample, resulting in different $T_{1/2}$ values. A modeling

Table 2 Pharmacokinetic parameters

	0.005 mg/kg (*n* = 4)	0.02 mg/kg (*n* = 6)	0.04 mg/kg (*n* = 6)	0.08 mg/kg (*n* = 6)	0.12 mg/kg (*n* = 6)	0.24 mg/kg (*n* = 6)	0.48 mg/kg (*n* = 6)
Plasma							
$AUC_{0\text{-}25\ h^*}$, h*ng/ml	18.58 ± 3.05	78.65 ± 7.96	154.50 ± 17.78	374.64 ± 37.12	470.27 ± 89.88	1597.39 ± 267.44	3065.25 ± 656.90
AUC_{inf}, h*ng/ml	18.55 ± 3.00	78.63 ± 7.96	154.57 ± 17.81	375.52 ± 37.65	470.79 ± 90.29	1600.39 ± 267.48	3070.10 ± 657.98
$AUC_{las}t$, h*ng/ml	18.25 ± 3.05	78.04 ± 7.85	153.72 ± 17.55	374.64 ± 37.12	468.95 ± 91.04	1597.39 ± 267.44	3065.25 ± 656.90
CL, L/h	12.54 ± 1.63	11.36 ± 0.68	13.40 ± 1.89	10.09 ± 0.78	12.46 ± 3.37	7.75 ± 1.48	8.12 ± 2.48
C_{max}, ng/ml	11.6 ± 1.75	42.2 ± 4.11	86.6 ± 17.4	196 ± 20.3	278 ± 42.6	855 ± 82.3	1970 ± 391
ke, l/h	0.43 ± 0.04	0.32 ± 0.07	0.25 ± 0.04	0.18 ± 0.03	0.24 ± 0.09	0.17 ± 0.03	0.19 ± 0.02
MRT, h	1.21 ± 0.12	1.70 ± 0.38	1.83 ± 0.81	1.91 ± 0.16	1.54 ± 0.34	1.78 ± 0.29	1.80 ± 0.24
T_{max}, h	1.0 (1.0–1.0)	1.0 (1.0–1.0)	1.0 (1.0–1.0)	1.0 (1.0–1.0)	1.0 (1.0–1.0)	1.0 (0.7–1.0)	1.0 (1.0–1.0)
Vd, L	29.35 ± 5.67	36.70 ± 6.72	54.09 ± 12.03	57.80 ± 9.04	55.98 ± 17.99	47.84 ± 15.32	44.51 ± 16.22
$T_{1/2}$, h	1.61 ± 0.14	2.25 ± 0.44	2.78 ± 0.41	3.97 ± 0.56	3.20 ± 0.87	4.24 ± 0.75	3.75 ± 0.34
Urine							
$Ae_{0\text{-}25\ h}^{\#}$, mg	0.058 ± 0.015	0.311 ± 0.036	0.450 ± 0.307	1.161 ± 0.353	1.621 ± 0.396	3.869 ± 1.517	6.099 ± 2.424
$Ae_{0\text{-}49\ h}^{\#}$, mg	0.058 ± 0.016	0.313 ± 0.037	0.455 ± 0.308	1.170 ± 0.356	1.633 ± 0.401	3.898 ± 1.530	6.150 ± 2.440
CLr, L/h	3.115 ± 0.572	3.957 ± 0.364	2.924 ± 1.942	3.080 ± 0.809	3.493 ± 0.867	2.380 ± 0.631	1.966 ± 0.545
$Fe_{0\text{-}49\ h}^{\#}$, %	25.331 ± 6.394	35.235 ± 4.080	21.727 ± 14.243	30.818 ± 8.068	29.245 ± 7.618	31.460 ± 9.153	25.658 ± 8.754

All data were described as "mean ± standard deviation," except Tmax was described as "median (range)"

Ae amount of drug excreted in urine, *AUC* area under the curve, *CL* clearance, C_{max} maximal concentration, *CLr* renal clearance, *Fe* excreted fraction, *ke* elimination rate constant, *MRT* mean residence time, T_{max} time to peak drug concentration, $T_{1/2}$ half-life, *Vd* distribution volume

*The dose group of 0.005 mg/kg in the pre-phase was $AUC_{0\text{-}24\ h}$

#The urine pharmacokinetic parameters of the dose group of 0.005 mg/kg in the pre-phase were $Ae_{0\text{-}24\ h}$, $Ae_{0\text{-}48\ h}$, and $Fe_{0\text{-}48\ h}$

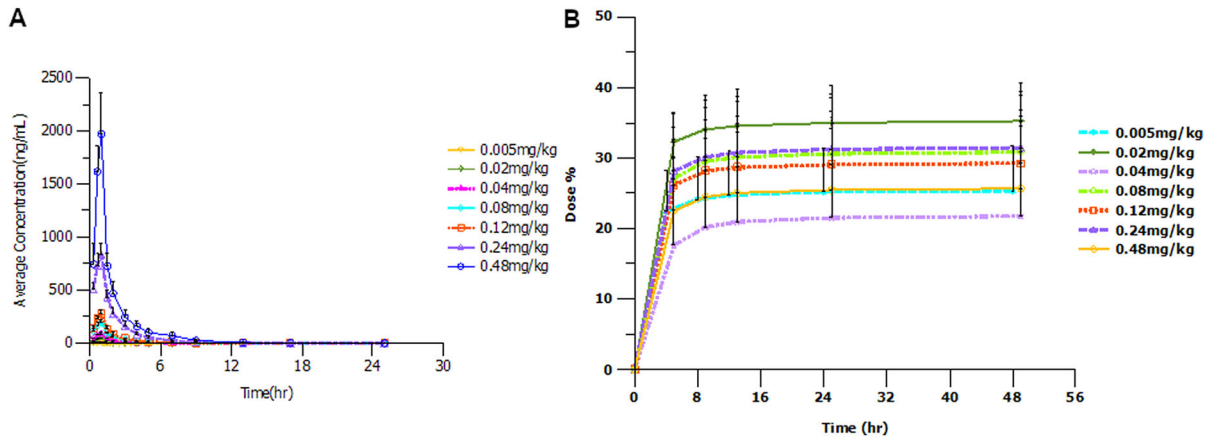

Fig. 2 **A** Mean time–concentration curves. **B** Mean urine cumulative excretion curves

Table 3 Relationships between pharmacokinetic parameters and doses

PK parameter	Point estimate	90% confidence interval	Criterion
Total dosage			
$AUC_{0\text{-}25\ h}$	1.108	1.063, 1.152	0.954, 1.046
AUC_{last}	1.111	1.067, 1.156	0.954, 1.046
AUC_{inf}	1.108	1.064, 1.153	0.954, 1.046
C_{max}	1.115	1.065, 1.164	0.941, 1.058
Dosage per kg			
$AUC_{0\text{-}25\ h}$	1.135	1.095, 1.176	0.954, 1.046
AUC_{last}	1.139	1.099, 1.180	0.954, 1.046
AUC_{inf}	1.136	1.096, 1.177	0.954, 1.046
C_{max}	1.145	1.103, 1.187	0.941, 1.058

AUC area under the curve, C_{max} maximal concentration, *PK* pharmacokinetic

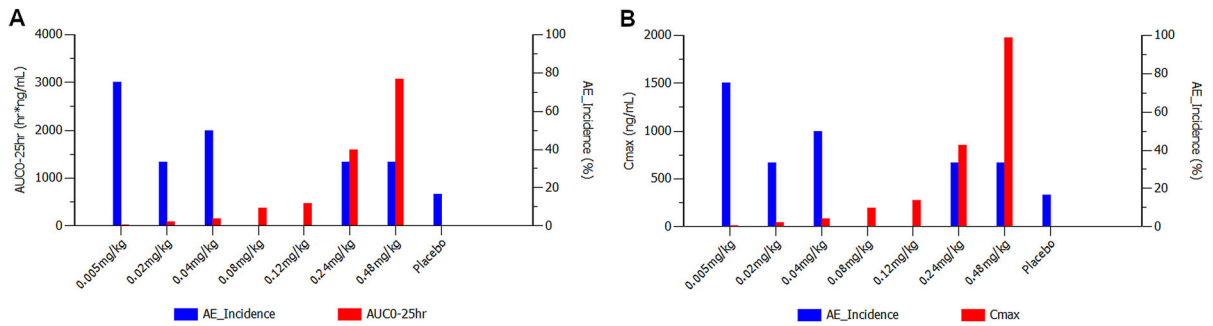

Fig. 3 Relationships between adverse event rates and kukoamine B mesylate exposure (**A**) and maximal concentration (**B**)

study proposed a simulation model for calculating the optimal dose of KB in patients with sepsis [13], but these results are theoretical at best and actual observations remain the best course. Another simulation study proposed that single dosing of KB would be recommended for clinical trials [14].

This study has limitations. The pharmacokinetic data collected represented the best case in healthy subjects and did not include variability due to patient covariates. Due to the small sample size, the safety profile needs to be further confirmed.

CONCLUSIONS

In this randomized, double-blind, placebo-controlled, single-dose phase I trial, single-dose KB demonstrated favorable safety and tolerability in healthy subjects at the dose level of 0.005–0.48 mg/kg. KB exhibited a non-linear pharmacokinetic profile with a half-life of about 1.61–4.24 h and was mainly distributed in plasma. The urinary excretion of KB decreased significantly about 8 h after administration. The main metabolic pathways of KB were bimethylation, carbonylation, *N*-desalkylation and cysteamine binding. The safety and efficacy of KB will be further investigated in patients with sepsis.

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Declarations

Conflict of interest. Hongzhong Liu, Qian Zhao, Yuping Yuan, Zhenlei Wang, Teng Wang, Wei Tian, Wen Zhong, Ji Jiang, and Pei Hu declare no conflicts of interest. Shuai Chen, Kai Kong, and Chunyan Jin are full-time employees of Tianjin Chasesun Pharmaceutical Co., Ltd.

Ethical Approval. The study was performed in accordance with the International Conference on Harmonization Good Clinical Practice guidelines and ethical principles that have their origin in the Declaration of Helsinki of 1964 and its later amendments. This study was approved by the Ethics Review Board of Peking Union Medical College Hospital (approved no. 2014L01029). All participants provided written informed consent before enrollment.

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