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CLINICAL THERAPEUTICS

A First-in-Human Safety, Tolerability, and Pharmacokinetics Study of Benapenem in Healthy Chinese Volunteers

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ABSTRACT The objective of this trial was to investigate the safety, tolerability, and pharmacokinetics (PK) of benapenem administered by single or multiple intravenous infusions in healthy Chinese volunteers. The trial was divided into 3 parts. In part A, 94 subjects were enrolled in a double-blind, placebo-controlled, sequential-ascending-single-dose study. The subjects were randomly assigned to groups receiving placebo or benapenem for injection at doses of 62.5, 125, 250, 500, 1,000, 2,000, or 3,000 mg. The effects of intravenous infusion time on the subjects of 250-, 500-, and 1,000-mg groups were explored. In part B, 12 subjects were enrolled in a single-dose PK study under fasting conditions and received 250, 500, or 1,000 mg of benapenem for injection. In part C, 36 subjects were given 250, 500, and 1,000 mg of benapenem for injection once daily for 7 consecutive days. The results showed that benapenem for injection was well tolerated during the studies. The major observed adverse events were mild, and all were resolved spontaneously without any medical intervention. Benapenem was mainly excreted through the kidneys in the form of parent molecule and metabolites. The PK and safety profiles of benapenem in healthy Chinese volunteers support its once-daily dosing in future clinical investigations. (Part A, part B, and part C have been registered at ClinicalTrials.gov under identifiers NCT03588156, NCT03578588, and NCT03570970, respectively.)

KEYWORDS benapenem, healthy Chinese volunteers, multiple dose, pharmacokinetics, safety, single dose, tolerance

Carbapenems (Fig. 1A) are the most potent class β-lactam antibiotics, with a relatively broad spectrum of activity against Gram-positive and Gram-negative bacteria, including aerobic and anaerobic bacteria (1). Carbapenems are stable to almost all β-lactamases, including AmpC β-lactamases and extended-spectrum β-lactamases (ESBLs), which makes them have relatively low drug resistance (2). As clinical use increases, the drug resistance rate is gradually growing (3–6). There are 7 carbapenem antibiotics approved by authority agents, and they are imipenem, panipenem, meropenem, ertapenem, biapenem, doripenem, and tipipepenan ester. Except for ertapenem, all six of the other carbapenems have short half-lives (approximately 1 h) and require 2 to 4 intravenous (i.v.) administrations daily in the clinic. Ertapenem has a relatively long half-life (about 4 h) because of a high protein-binding percentage (85 to ~95%) and can be clinically administered once daily (7). Therefore, it is urgent that we develop new carbapenem antibiotics with better pharmacokinetic characteristics to meet clinical needs.

Recently, Sihuan Pharmaceuticals Co., Ltd., Shandong, China, has designed and synthesized a new series of carbapenems. Among them is benapenem (Fig. 1B), designed to amalgamate a methyl-benzylsulfonamide moiety to the classical structure of carbapenems to increase the molecular weight and lipophilicity. Preclinical pharma-

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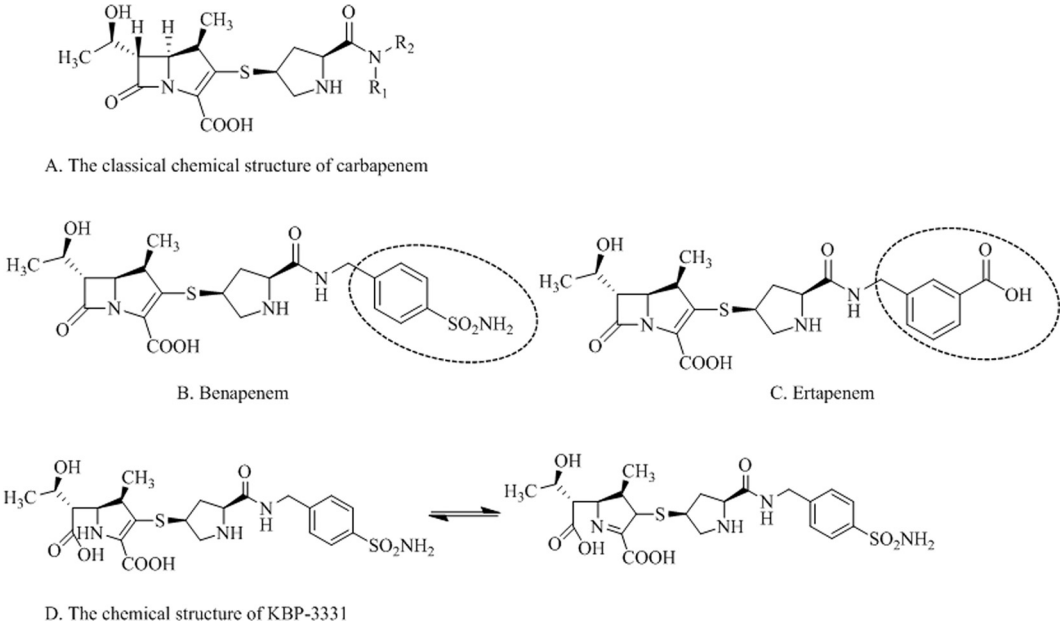

FIG 1 Chemical structure of benapenem and KBP-3331.

cological results (unpublished data provided by Sihuan Pharmaceuticals) showed that benapenem was a time-dependent bactericidal drug and had an antibacterial spectrum similar to that of other carbapenems, like meropenem, imipenem, and ertapenem (Fig. 1C), with a 50% minimum inhibitory concentration (MIC_{50}) less than 1 mg/liter to most bacteria *in vitro*. The ratio of minimum bactericidal concentration (MBC) to MIC was less than 4× for most bacteria, suggesting that benapenem is a classical bactericidal agent. Additionally, benapenem displayed good pharmacokinetic profiles in animals. The clearance (CL) and elimination half-life ($t_{1/2}$) of benapenem in rats is 0.18 liter/h/kg of body weight and 2.3 h, respectively. Moreover, benapenem exhibited higher protein binding at lower concentrations in human plasma protein, ranging from 41.5% at total concentrations of 2,000 μg/liter to 92% at concentrations of 0.002 μg/liter. To further understand the safety and PK profiles of benapenem, a first-in-human phase I study was conducted in a Chinese population.

RESULTS

Safety and tolerability. There were no serious adverse events (AEs) observed in this trial. In part A, a total of 11 subjects (14.86%) in the active group reported 20 AEs, and 2 of the 20 subjects (10.00%) in the placebo group reported 2 AEs. The AEs possibly related to drug treatment are summarized in Table 1. All these AEs were mild (Common Terminology Criteria for AEs [CTCAE; v4.02; http://evs.nci.nih.gov/ftp1/CTCAE/About.html] grade 1) and relieved spontaneously without any treatment intervention. Four AEs in 3 subjects (4.05%) in the active group and 2 AEs in 2 subjects (10.00%) in the placebo group were determined to be relevant to drug administration. The AEs were white blood cells (WBC) decreasing (2 cases), alanine aminotransferase (ALT; 2 cases) or aspartate transaminase (AST; 1 case) increasing, and erythema (1 case). Each of the two AEs of WBC decreasing were found in the placebo group (changing from 4.5×10^9 to 3×10^9/liter) and the group receiving 500 mg for 60-min (changing from 3.2×10^9 to 2.8×10^9/liter). Each of two AEs of ALT increasing was found at the 125-mg dose for the 30-min injection group (changing from 15 to 53 IU/liter) and 3,000 mg for the 60-min injection group (changing from 8 to 67 IU/liter). One AE of AST increasing was found in the same person, with ALT increasing at the 3,000-mg dose for the 60-min injection group (changing from 15 to 51 IU/liter). One AE of erythema was found in the injection group receiving 500 mg for 60 min.

TABLE 1 Summary of possible treatment-related adverse events at each dose level

	Part A[a]												Part C			
AE	Total ($n = 94$)	Placebo ($n = 20$)	62.5 mg/ 30 min ($n = 6$)	125 mg/ 30 min ($n = 6$)	250 mg/ 30 min ($n = 8$)	500 mg/ 60 min ($n = 8$)	500 mg/ 30 min ($n = 8$)	1,000 mg/ 60 min ($n = 8$)	1,000 mg/ 30 min ($n = 8$)	2,000 mg/ 60 min ($n = 8$)	2,000 mg/ 30 min ($n = 8$)	3,000 mg/ 60 min ($n = 6$)	Total ($n = 36$)	250 mg ($n = 12$)	750 mg ($n = 12$)	1,000 mg ($n = 12$)
WBC decreased	2	1 (5%, F)	0	0	0	1 (12.5%, F)	0	0	0	0	0	0				
ALT increased	2	0	0	1 (16.7%, M)	0	0	0	0	0	0	0	1 (16.7%, M)	4	1 (8.3%, F)	0	3 (25%, M)
AST increased	1	0	0	0	0	0	0	0	0	0	0	1 (16.7%, M)	1	1 (8.3%, F)	0	0
Erythema	1	1 (5%, M)	0	0	0	0	0	0	0	0	0	0				

[a]M, male; F, female.

TABLE 2 Pharmacokinetic parameters (means ± SD) of benapenem following a single dose or 7 days of multiple doses

Study and dose	$t_{1/2}$ (h)	T_{max} (h)	C_{max} (mg/liter)	AUC_{0-t} (h·mg/liter)	AUC_{0-inf} (h·mg/liter)	V_z (liter)	CL (liter/h)	MRT_{0-inf} (h)
Single ascending dose								
250 mg ($n = 12$)	6.79 ± 0.71	0.48 ± 0.07	55.65 ± 13.20	297.93 ± 43.96	311.05 ± 42.18	7.95 ± 0.94	0.82 ± 0.11	8.26 ± 1.01
500 mg ($n = 12$)	6.81 ± 0.81	0.50 ± 0.00	96.51 ± 9.13	555.62 ± 65.79	566.30 ± 64.56	8.71 ± 0.88	0.89 ± 0.10	8.24 ± 0.96
1,000 mg ($n = 12$)	6.67 ± 0.81	0.50 ± 0.00	165.00 ± 17.79	948.12 ± 120.83	960.12 ± 121.52	10.08 ± 1.01	1.06 ± 0.14	8.00 ± 1.05
Multiple ascending doses								
250 mg ($n = 12$)								
Day 1	6.53 ± 0.84	0.50 ± 0.00	49.04 ± 5.30	260.36 ± 33.00	280.06 ± 39.16	8.48 ± 0.97	0.91 ± 0.13	7.95 ± 1.16
Day 7	6.06 ± 0.82	0.50 ± 0.00	50.04 ± 4.84	281.96 ± 45.62	283.53 ± 46.02	8.33 ± 0.86	0.96 ± 0.14	7.67 ± 1.08
500 mg ($n = 12$)								
Day 1	6.65 ± 0.88	0.50 ± 0.00	97.87 ± 10.09	532.45 ± 85.37	575.39 ± 99.94	8.47 ± 1.25	0.89 ± 0.17	8.09 ± 1.09
Day 7	6.16 ± 0.65	0.50 ± 0.00	105.18 ± 10.56	589.98 ± 80.86	592.71 ± 82.24	8.10 ± 0.86	0.92 ± 0.11	7.66 ± 1.00
1,000 mg ($n = 12$)								
Day 1	6.46 ± 0.88	0.50 ± 0.00	161.83 ± 15.02	851.94 ± 88.16	913.50 ± 109.75	10.22 ± 0.91	1.11 ± 0.15	7.75 ± 1.02
Day 7	6.33 ± 0.63	0.50 ± 0.00	180.83 ± 16.65	996.79 ± 151.67	1002.23 ± 154.72	9.87 ± 0.79	1.09 ± 0.15	7.60 ± 0.81

No subjects in part B were observed to have any AEs, and all laboratory examination results were in the normal range. In part C, 8 subjects (22.22%) in the three dose groups had a total of 15 AEs. Five AEs, which were increasing ALT (4 cases) or AST (1 case), in 4 subjects (11.11%) were determined to be related to the study drug. One of four AEs of ALT increasing was found in the 250-mg group (changing from 18 to 60 IU/liter), and three were found in the 1,000-mg group (changing from 25 to 56, 35 to 79, and 16 to 57 IU/liter, respectively). One AE of AST increasing was found in the same person with ALT increasing in 250-mg group (changing from 18 to 60 IU/liter).

In part A, we set up a study to evaluate the effect of intravenous infusion time, 30 min and 60 min, on the safety and tolerability in three different doses: 500 mg, 1,000 mg, and 2,000 mg. The results showed that the infusion rate had no effect on the subject's tolerability.

Single-dose pharmacokinetics. After a single-dose administration of benapenem at 250 mg, 500 mg, and 1,000 mg, benapenem had C_{max} (maximum measured serum concentration), AUC_{0-t} (area under the concentration-time curve to the last measureable concentration), and AUC_{0-inf} (area under the concentrate-time curve to infinity) ranging from 55.65 to 165 mg/liter, 297.93 to 984.12 h·mg/liter, and 331.05 to 960.12 h·mg/liter, respectively (Table 2), while the principle metabolite KBP-3331 had C_{max}, AUC_{0-t}, and AUC_{0-inf} ranging from 1.86 to 7.12 mg/liter, 15.96 to 51.34 h·mg/liter, and 16.31 to 51.84 h·mg/liter, respectively (Table 3). These data suggest that benapenem and KBP-3331 increased in proportion to dose, showing a linear pharmacokinetic feature (Fig. 2). The median T_{max} (the time after administration that the maximum

TABLE 3 Pharmacokinetics of KBP-3331 (a metabolic product of benapenem) following a single dose or 7 days of multiple doses (means ± SD)

Study and dose	$t_{1/2}$ (h)	T_{max} (h)	C_{max} (mg/liter)	AUC_{0-t} (h·mg/liter)	AUC_{0-inf} (h·mg/liter)	MRT_{0-inf} (h)
Single ascending dose						
250 mg ($n = 12$)	7.32 ± 0.77	0.48 ± 0.07	1.86 ± 0.52	15.96 ± 2.48	16.31 ± 2.50	10.22 ± 0.18
500 mg ($n = 12$)	7.37 ± 0.78	0.52 ± 0.07	3.41 ± 0.54	28.85 ± 3.81	29.24 ± 3.82	10.00 ± 1.12
1,000 mg ($n = 12$)	7.12 ± 0.65	0.50 ± 0.00	7.12 ± 1.21	51.34 ± 7.01	51.84 ± 7.21	8.75 ± 0.94
Multiple ascending doses						
250 mg ($n = 12$)						
Day 1	7.00 ± 0.99	0.50 ± 0.00	1.52 ± 0.22	12.41 ± 1.55	13.74 ± 1.97	9.72 ± 1.48
Day 7	6.71 ± 1.00	0.50 ± 0.00	1.34 ± 0.22	12.44 ± 2.92	12.76 ± 2.93	9.35 ± 1.48
500 mg ($n = 12$)						
Day 1	6.99 ± 1.01	0.52 ± 0.07	3.53 ± 0.58	27.73 ± 5.90	30.71 ± 7.10	9.64 ± 1.49
Day 7	6.74 ± 0.74	0.50 ± 0.00	3.73 ± 0.66	30.71 ± 6.23	31.08 ± 6.31	9.20 ± 1.21
1,000 mg ($n = 12$)						
Day 1	6.69 ± 0.88	0.50 ± 0.00	8.24 ± 1.02	53.41 ± 9.20	58.19 ± 11.10	8.70 ± 1.18
Day 7	6.67 ± 0.68	0.50 ± 0.00	9.35 ± 0.89	65.50 ± 12.70	66.08 ± 12.93	8.65 ± 1.03

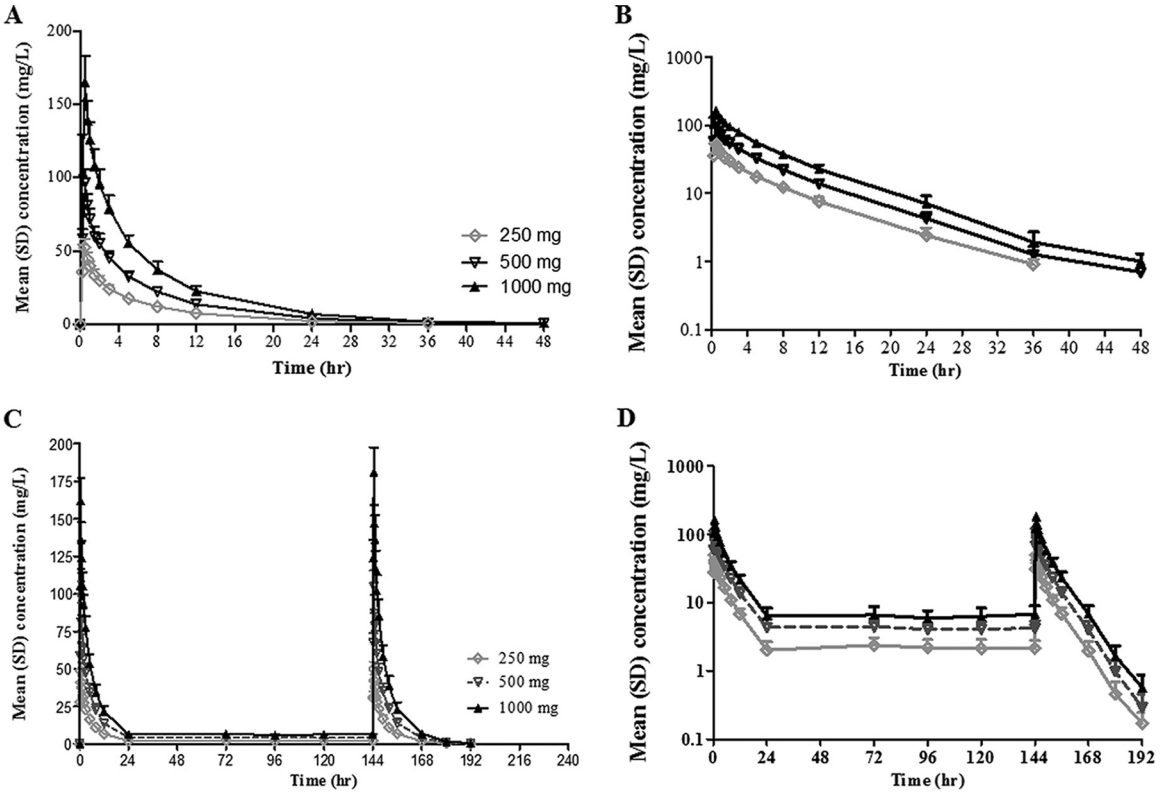

FIG 2 Mean plasma concentration-versus-time profiles of benapenem following a single dose or 7 days of multiple doses. (A and B) Two hundred fifty mg, 500 mg, and 1,000 mg benapenem in single-ascending-dose study, representing part B of the study. Panel A is linear scale, and panel B is $\log_{10}$ scale. (C and D) Two hundred fifty mg, 500 mg, and 1,000 mg benapenem in multiple-ascending-dose study, representing part C of the study. Panel C is linear scale, and panel D is $\log_{10}$ scale.

serum concentration is achieved) for benapenem was 0.5 h. Mean $t_{1/2}$ and $MRT_{0\text{-inf}}$ (mean retain time to infinity) of benapenem after a single dose ranged from 6.67 to 6.81 h and 8.00 to 8.26 h, respectively. Mean V_z (the apparent volume of distribution during the terminal phase) and clearance (CL) for benapenem ranged from 7.95 to 10.08 liters and 0.82 to 1.06 liters/h (Table 2). Exposure of the metabolite KBP-3331 was approximately 5.3% of that of benapenem. The $t_{1/2}$ and $MRT_{0\text{-inf}}$ of KBP-3331 after a single dose ranged from 7.12 to 7.37 h and 8.75 to 10.22 h, respectively (Table 3).

As shown in Table 4 and Fig. 3, the cumulative urinary excretion of benapenem at 250, 500, and 1,000 mg for single administration was 41.24%, 44.42%, and 39.64%, respectively, and renal clearance was 0.35, 0.41, and 0.42 liter/h, respectively. The cumulative urinary excretion of benapenem metabolite KBP-3331 was 30.89%, 31.22%, and 26.60% and the renal clearance rates were 5.12, 5.77, and 5.47 liter/h after 250, 500, and 1,000 mg of benapenem administration, respectively. Thus, the cumulative urinary excretion of benapenem and KBP-3331 was 72.13%, 75.64%, and 66.24%, corresponding to administered doses of 250 mg, 500 mg, and 1,000 mg, respectively. These results

TABLE 4 Pharmacokinetics of benapenem and KBP-3331 in urine and feces in 48 h following a single dose (means ± SD)

Dose	Value(s) for:				
	Urine				
	Cumulative excretion (%)		Renal clearance (liters/h)		
	Benapenem	KBP-3331	Benapenem	KBP-3331	Cumulative excretion (%) of feces
250 mg ($n = 12$)	41.24 ± 7.77	30.89 ± 6.71	0.35 ± 0.08	5.12 ± 1.38	
500 mg ($n = 12$)	44.42 ± 11.31	31.22 ± 7.53	0.41 ± 0.14	5.77 ± 1.96	0.007 ± 0.015
1,000 mg ($n = 12$)	39.64 ± 8.23	26.60 ± 5.20	0.42 ± 0.09	5.47 ± 1.33	

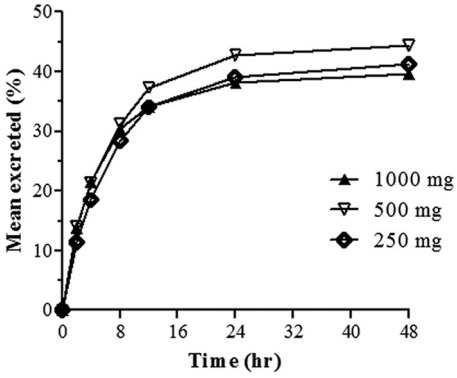

FIG 3 Mean accumulated amount of benapenem excreted in 48 h following a single dose.

suggest that excretion of benapenem in the human body was mainly through the kidney after a single dose. The cumulative excretion of feces after single administration of 500 mg benapenem was only 0.007%, and the cumulative excretion of KBP-3331 was equivalent to 0.539% of that of benapenem. The results suggest that excretion of benapenem by feces was very low and could be negligible.

Multiple-dose pharmacokinetics. After the multiple-dose administration of benapenem at 250 mg, 500 mg and 1,000 mg once daily for 7 consecutive days, the mean C_{max}, $AUC_{0\text{-}t}$, and $AUC_{0\text{-inf}}$ for benapenem increased in proportion to dose, which was similar to that of the single-dose administration. The mean T_{max} for benapenem was 0.5 h. Mean $t_{1/2}$ and $MRT_{0\text{-inf}}$ of benapenem after the multiple-dose administration for consecutive 7 days ranged from 6.06 to 6.65 h and 7.60 to 8.09 h, respectively. Mean V_z for benapenem ranged from 8.10 to 10.22 liters. The C_{max}, $AUC_{0\text{-}t}$, and $AUC_{0\text{-inf}}$ for KBP-3331 after the first dose of benapenem ranged from 1.34 to 8.24 mg/liter, 12.41 to 53.41 h·mg/liter, and 13.74 to 58.19 h·mg/liter, respectively. The C_{max}, $AUC_{0\text{-}t}$, and $AUC_{0\text{-inf}}$ for KBP-3331 after the administration on day 7 ranged from 1.34 to 9.35 mg/liter, 12.44 to 65.50 h·mg/liter, and 12.76 to 66.08 h·mg/liter, respectively, which indicates that after the multiple-dose administration of benapenem, the strict linear relationship of KBP-3331 was not established. The exposure of the metabolite KBP-3331 to benapenem in the multiple dose was approximately the same as that of the single dose, with the ratio ranging from 4.8% to 6.5%. The $t_{1/2}$ and $MRT_{0\text{-inf}}$ of KBP-3331 after multiple-dose administration ranged from 6.67 to 7.00 h and 8.65 to 9.72 h, respectively (Table 3).

After the consecutive 4 days of administration, the drug concentration had reached a steady state in the body and the concentrations were basically the same (Fig. 2). After stabilization, the body accumulative index (the mean ratio of $AUC_{0\text{-inf}}$ on day 7 to that on day 1) was about 1.07. The curves after the 1st and 7th administrations were basically similar, suggesting the metabolism of the drug *in vivo* did not change with the extension of time.

DISCUSSION

The safety of carbapenems has been proved to be comparable to or better than that of other β-lactams. The side effects of carbapenems are mainly infusion site complications, diarrhea, nausea, and vomiting (8). In this trial, the AEs possibly related to drug administration were erythema (1 case reported, 5%) and changes of blood biochemical indicators, such as ALT, AST, and WBC (Table 1). All of these AEs were mild and relieved spontaneously without any treatment intervention. In addition, the i.v. infusion rate (30 min or 60 min), which is regularly used for other carbapenems, had no effect on the subject's tolerability. Therefore, both the ascending-single-dose and multidose administrations of benapenem were well tolerated, and no safety concerns were identified.

In the single-ascending-dose study, about half an hour after drug administration, the C_{max} of benapenem at doses of 250, 500, and 1,000 mg ranged from 55 to ~165 mg/

liter, reaching the MIC_{50} (less than 1 mg/liter) of benapenem to most bacteria *in vitro*. For instance, based on the MIC_{90} (less than 0.5 mg/liter) of benapenem for *Enterobacteriaceae*, after a single dose of 250, 500, and 1,000 mg, the effective bactericidal concentration of benapenem in plasma could last 36 h. Moreover, for 250-, 500-, and 1,000-mg multidose administration of benapenem, the steady-state plasma concentrations were all above 1 mg/liter. The PK results of the trial showed that benapenem had a longer serum half-life (6 to ~7.3 h) than market-available carbapenems, which have a half-life of 1 to ~4 h (9, 10). The short half-life of carbapenems, like imipenem, meropenem, and doripenem, requires 2 or 4 times daily administration to achieve the desired MIC (3, 4, 11). Therefore, the relatively long half-life time and superior pharmacokinetic-pharmacodynamic profile of benapenem would support its once-daily use in clinics.

Older carbapenems, such as imipenem, were often susceptible to degradation by the enzyme dehydropeptidase-1 (DHP-1), located in renal tubules, and required coadministration with a DHP-1 inhibitor such as cilastatin (12). In this study, about 44% of benapenem prototype was excreted renally. We proposed that benapenem was relatively stable to DHP-1. The principal metabolite of benapenem is KBP-3331, which is the open-ring hydrolysis product. The open-ring hydrolysis product is also the principle metabolite of ertapenem (13). Although KBP-3331 has no bactericidal activity, no other main metabolites (>10%) were found besides KBP-3331; thus, the PK of KBP-3331 was also investigated in this human study. The PK of KBP-3331 both in the single-dose and multiple-dose administration has a profile similar to that of benapenem.

In conclusion, this study demonstrated the safety and tolerability of benapenem after a single and multiple intravenous injections in healthy subjects. The pharmacokinetics of benapenem showed a relatively long half-life and was correlated with its pharmacodynamic effects, supporting its once-daily i.v. administration in clinics.

MATERIALS AND METHODS

The trial was conducted in accordance with the Declaration of Helsinki (World Medical Association), Good Clinical Practice (GCP) guidelines, and the laws and regulations of China. The study has been approved (approval no. 2012L02697) by the China Food and Drug Administration (CFDA). The study protocol and informed consent forms were approved by the Independent Ethics Committee and the Institutional Review Board of Peking University First Hospital.

Trial design and drug administration. As shown in Fig. 4, the trial was divided into three parts: part A, a single-ascending-dose study to assess safety and tolerability of benapenem; part B, a single-dose study to observe the PK profiles of benapenem and its major metabolite; and part C, a multiple-dose study to observe the tolerability and PK profiles of benapenem and its major metabolite.

Part A was a randomized, double-blind, placebo-controlled, single-ascending-dose safety and tolerability study involving 7 doses of benapenem (62.5, 125, 250, 500, 1,000, 2,000, and 3,000 mg). In total, 98 healthy subjects (male/female ratio, 1:1) were randomly assigned to 1 of 10 cohorts. As shown in Table 5, 2 subjects received a placebo and six or eight subjects received single doses of benapenem via intravenous injection. Test drugs or placebo was dissolved in saline with a total volume of 100 ml and administered to subjects who were fasted overnight for at least 12 h. To ensure the subject's safety and evaluate the effect of infusion rate on tolerability, two infusion times were designed. The subjects in the three dose groups, 62.5 mg, 125 mg, and 250 mg, were infused only for 30 min, and the 3,000-mg group was infused for 60 min only. If the first several subjects in the groups of 500 mg could tolerate 60 min of infusion with study drugs, then the remaining subjects would receive drug administration with the infusion time decreased to 30 min, so as to evaluate the effect of the infusion rate on tolerability. The same protocol as that for the 500-mg group was performed in 1,000-mg and 2000-mg groups.

Part B was a single-dose pharmacokinetic study, with a randomized, open-label, three-phase, crossover design. Twelve healthy adult volunteers (male/female ratio, 1:1) were enrolled and randomly assigned to three groups (4 subjects/group, 2 males and 2 females), B1, B2, and B3. The subjects in each group only received a single dose of benapenem for injection: 250 mg, 500 mg, or 1,000 mg. In the first phase, the subjects in the B1 group were given 250 mg benapenem for injection, and then B2 and B3 would be given 500 mg and 1,000 mg benapenem for injection, respectively. After a 7-day washout period, subjects in B1, B2, and B3 groups were given single doses of 500 mg, 1,000 mg, and 250 mg benapenem, respectively, for injection in the second phase. In the third phase, B1, B2, and B3 were administered a single dose of 1,000 mg, 500 mg, and 250 mg benapenem for injection after a 7-day washout period.

Part C was an open-label and multiple-dose study. Thirty-six healthy subjects were enrolled and randomly assigned into 3 groups (12 subjects/group; male/female ratio of 1:1). Each subject was given (i.v.) only one dose of study drug, 250 mg, 500 mg, or 1,000 mg, once daily for 7 consecutive days. The study was first conducted in subjects of the 250-mg group. After observation was completed and the

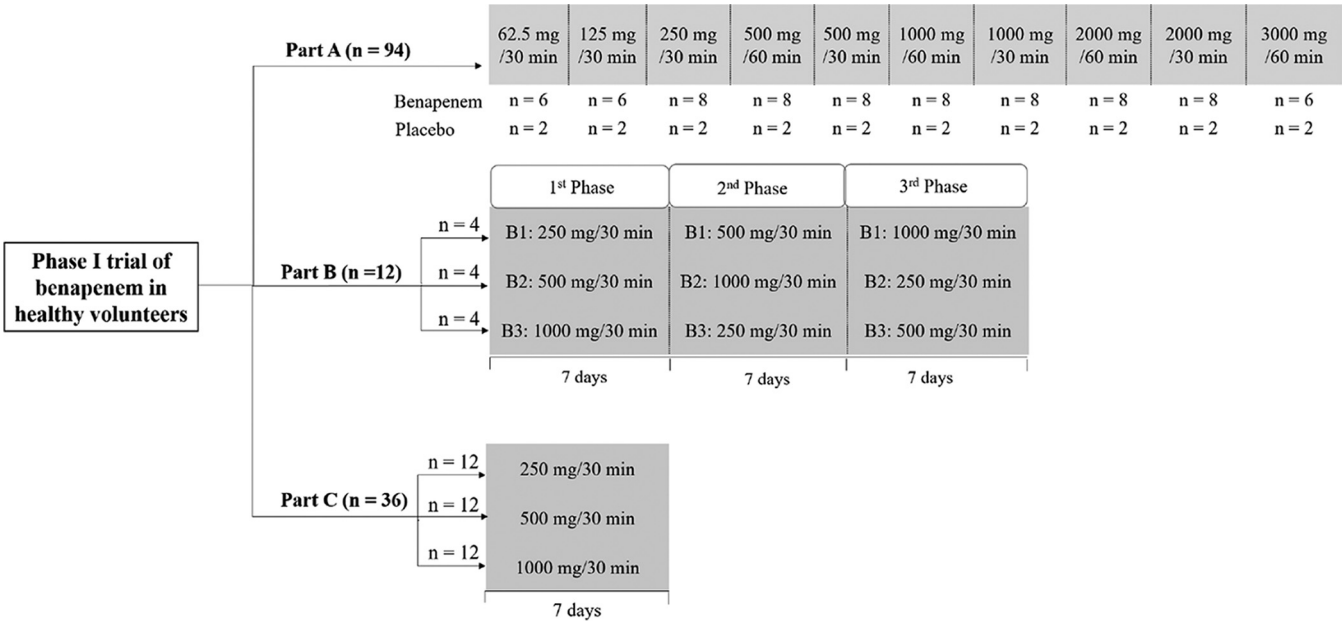

FIG 4 Phase I trial design of benapenem in healthy Chinese volunteers. Part A, a single-ascending-dose study to assess safety and tolerability of benapenem; part B, a single-dose study to evaluate the PK profiles of benapenem and its major metabolites; part C, a multiple-dose study to assess the tolerability and PK profiles of benapenem and its major metabolites.

safety and tolerability of the dose of 250 mg confirmed, the study was conducted on the subjects of the 500-mg group and last in the subjects of the 1,000-mg group.

Subjects and demographic characteristics. Healthy male and female Chinese subjects, aged from 18 to 45 years with body mass index (BMI) values of 19 to ~26 kg/m^2 (body weight, ≥50 kg), were eligible to participate in the study. The eligible subjects were determined by medical history, physical examination, 12-lead safety electrocardiogram (ECG), and clinical laboratory tests. Exclusion criteria included volunteers who had a history of specific allergies (such as allergic dermatitis, asthma, etc.) or a history of drug allergies, especially those who were allergic to β-lactam drugs and drug accessories. No pregnant or lactating women were included. All subjects provided written informed consent. The demographic data of the subjects in the single-ascending-dose, single-dose PK, and multiple-dose PK studies are presented in Table 5.

Safety assessments. Safety and tolerability were evaluated on the basis of AEs, physical examination, vital signs, clinical laboratory tests, and 12-lead ECG data. Subjects were monitored carefully throughout each dosing period for AEs. The relationship of AEs to the study drug was evaluated by the

TABLE 5 Baseline demographics

Parameter	Value(s) for part: A		B	C
	Benapenem (*n* = 74)	Pooled placebo (*n* = 20)	B (*n* = 12)	C (*n* = 36)
Age (yr)				
Mean ± SD	29.21 ± 6.14	30.75 ± 6.93	28.16 ± 4.74	28.25 ± 6.33
Range	18~42	21~42	21~36	20~44
Height (cm)				
Mean ± SD	165.61 ± 7.67	166.20 ± 6.67	164.92 ± 8.22	165.48 ± 7.14
Range	149~180	153~178	154~181	152~181
Weight (kg)				
Mean ± SD	61.53 ± 7.03	59.90 ± 5.57	62.18 ± 7.74	61.41 ± 6.74
Range	50~77	50~70	52~78	50~75
BMI (kg/m^2)				
Mean ± SD	22.38 ± 1.35	21.65 ± 1.15	22.78 ± 1.22	22.36 ± 1.22
Range	19.50~24.50	19.10~23.70	20.80~24.00	19.70~23.90

investigator. Identification of the intensity of each AE was performed on the basis of the CTCAEs (v4.02) (http://evs.nci.nih.gov/ftp1/CTCAE/About.html).

Drug analysis. A liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed and validated to determine the concentration of benapenem and metabolite KBP-3331 in human plasma, urine, and feces samples. Plasma or urine samples (50 μl) were mixed with the internal standard working solution (benapenem; provided by XuanZhu Pharma, China), followed by 400 μl of water. The mixture was placed on a Titer plate shaker, vortexed for approximately 10 min, and centrifuged at 4,000 $\times$ *g* for 10 min. The supernatant (50 μl) was transferred to a 96-well plate, and 450 μl water was added. After that, the plate was vortexed on a plate shaker for 10 min for analysis. Feces sample (100 μl) was added into a 1.5-ml polypropylene tube and along with 330 μl of purified water. The tube was vortexed for 5 min and then was centrifuged at 14,000 $\times$ *g* for 5 min at 4°C. The supernatant (150 μl) was transferred to a 96-well plate, and 100 μl purified water was added and mixed for analysis. Chromatographic separation was performed with a reverse-phase column (ZORBAX Eclipse plus C_{18}; 5 μm [2.1-mm inner diameter by 50 mm]; Agilent, USA). Gradient elution was from mobile phase A (0.1% formic acid and 2 mM ammonium acetate in water) to mobile phase B (0.1% formic acid with 2 mM ammonium acetate in 95% acetonitrile).

An API 400 MS equipped with an electrospray ionization (ESI) source (Sciex, USA) was used for MS analysis. The analysis was operated in multiple reaction monitoring (MRM) modes under unit mass resolution in both the Q1 and Q3 mass analyzers. The ion pair mass-to-charge ratio for internal standards, benapenem, and its principal metabolism were 531.1/487.1, 525.1/481.1, and 543.1/499.1, respectively. Data acquisition was performed using Analyst software (version 1.5.2), and data processing used Watson LIMS software (version 7.2.0.02) and Microsoft Excel 2003.

The calibration ranges of the plasma, urine, and feces assays for benapenem and KBP-3331 were all linear ($r^2 \geq 0.999$). Calibrations of benapenem in plasma, urine, and feces were 0.60 to 300 μg/ml, 0.40 to 200 μg/ml, and 2 to 1,000 ng/ml, respectively. Calibrations of KBP-3331 in plasma, urine, and feces were 0.02 to 10 μg/ml, 0.40 to 200 μg/ml, and 20 to 1,0000 ng/ml, respectively. All samples were analyzed and met acceptance criteria for standard curve and quality control (QC) samples. The accuracy of the method was determined by comparing the mean measured concentrations with theoretical concentrations of each analyte in the QC samples. The deviations of the means from theoretical values did not exceed 12% for benapenem and KBP-3331. The precision was determined from the percent coefficient of variation (%CV) of the QC sample replicates at each concentration level. The %CV for benapenem and KBP-3331 ranged from 1.7% to 12.9%.

Pharmacokinetics. The blood samples were taken from the arm contralateral to the intravenous infusion. For single-dose PK analysis, blood samples were collected at 0 h (predose, within 1 h before dosing) and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, and 48 h after dosing. For multiple-dose PK analysis, blood samples on day 1 were collected at the same time points as those of the single-dose PK analysis until 24 h. On day 7, the sampling time points were 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, and 48 h after the last dosing. In addition, blood samples before dosing were collected on days 5, 6, and 7. Blood samples (4 ml) were drawn into lithium-heparinized tubes and were centrifuged. Plasma samples were separated within 30 min. Urine samples for single-dose PK analysis were collected before dosing (within 1 h before dosing) and over the time intervals of 0 to 4, 4 to 8, 8 to 12, 12 to 24, and 24 to 48 h postdosing. The feces samples were collected only from the 12 subjects in the 500-mg group in the single-dose PK study from the first day to 48 h after drug administration. The subjects were asked to empty stool before dosing. The time of specimen collection and the total weight of stool after mixing were recorded. All samples were stored at −70°C until analysis.

Both in nonclinical PK study and in toxicokinetics study, one the main metabolites of benapenem was identified as KBP-3331 (Fig. 1D), which is the open-ring hydrolysis product. The open-ring hydrolysis product is also the principle metabolite of ertapenem (13). Although KBP-3331 has no bactericidal activity, no other main metabolites (>10%) were found except KBP-3331; the PK of KBP-3331 was also investigated in this human study.

PK parameters were calculated using noncompartmental analysis with the Phoenix WinNonlin (6.3) (Pharsight Corp., Mountain View, CA). The PK parameters assessed included C_{max}, T_{max}, $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, $t_{1/2}$, $MRT_{0\text{-}inf}$, and V_z.

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