In Vitro and In Vivo Activity of Tebipenem Against ESBL-Producing *E. coli*

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Objective: *To determine in vitro and in vivo activity of tebipenem against ESBL-producing E. coli.*
Material and Method: *Minimum inhibitory concentration (MIC) of tebipenem against 100 clinical isolates of ESBL-producing E. coli was performed by broth micro-dilution technique. Blood and urine samples from 10 healthy male subjects before and after receiving 300 mg of tebipenem pivoxil 3 times a day for 2 consecutive days were determined for inhibitory and bactericidal titers against a clinical urinary isolate of ESBL-producing E. coli by disk diffusion method and broth micro-dilution method.*
Results: *MIC_{50} and MIC_{90} of tebipenem against ESBL-producing E. coli were both ≤0.06 mg/L with MIC range from ≤0.06 to 0.25 mg/L. The inhibition zones were observed around the disks inoculated with serum samples and urine samples collected from all study subjects after receiving tebipenem pivoxil for at least 1 hour and 5 hours, respectively. The inhibitory titer of 1:160 and bactericidal titer of 1:160 of serum samples were observed for at least one hour after ingestion of tebipenem pivoxil. Inhibitory titer of ≥1:640 and bactericidal titer of ≥1:640 of urine samples were observed after at least 14 hours after ingestion of tebipenem pivoxil. No subjects experienced side effects related to receiving tebipenem pivoxil.*
Conclusion: *Tebipenem is very active against ESBL-producing E. coli. Oral administration of tebipenem pivoxil 300 mg 3 times a day for two days was well tolerated, safe and induced high inhibitory and bactericidal activity in serum and urine. Tebipenem pivoxil could be an oral agent for effective therapy of ESBL-producing E. coli infections.*

Keywords: *Tebipenem, ESBL-producing E. coli, In vitro activity, In vivo activity*

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An emergence of bacterial resistance has become a public health threat over the past several decades[1]. Extended spectrum beta-lactamase (ESBL) producing gram-negative bacteria has been considered a major problem of antimicrobial resistance. A recent study conducted at Siriraj Hospital revealed that the prevalence of ESBL-producing gram-negative bacilli accounted for 30.1% of all gram-negative bacteria isolated from hospitalized patients with nosocomial infections[2]. ESBL-producing gram-negative bacteria were observed in 36% of outpatients with upper urinary tract infections[3]. A surveillance study conducted in healthy individuals living in Nan, Kanchanaburi, and Nakhon si Thammarat provinces revealed that approximately 30% to 50% of them were colonized with ESBL-producing pathogens in their gastrointestinal tracts[4]. Prior colonization with ESBL-producing enterobacteriaceae is one of the risk factors for developing ESBL infections[5]. Therefore, the infections caused by ESBL-producing bacteria are prevalent in hospital-acquired infections and they are expected to be a major problem in community-acquired infections in the near future.

ESBL-producing gram-negative bacteria are usually resistant to cephalosporins, aminoglycosides and fluoroquinolones, which are the first line antibiotics for therapy of gram-negative bacterial infections[6]. The most effective antibiotic for therapy of infections caused by ESBL-producing gram-negative bacteria is the carbapenem group including imipenem, meropenem, and doripenem[7]. However, the aforementioned carbapenems, which are available in Thailand, are parenteral agents and the patients who will receive such agents require hospitalization. Ertapenem is also a parenteral antibiotic of choice for therapy of ESBL-producing gram-negative bacterial infection and it can be given as a once daily dose. Therefore, an oral antibiotic that is effective for therapy of outpatients with ESBL-producing gram-negative bacterial infections is needed.

The oral antibiotics that contain antimicrobial activity against ESBL-producing gram-negative

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bacteria include nitrofurantoin[8], sitafloxacin[9], fosfomycin[8]. Nitrofurantoin is a member of a group of synthetic nitrofuran compounds. After oral administration, serum concentration of nitrofurantoin is very low or sometimes undetectable (≤1 µg/ml) with a standard dose of 100 mg four times daily. Nitrofurantoin has been approved by Thai FDA for treatment of acute cystitis. Fosfomycin has two formulations, oral and parenteral forms. Both formulations are available in Thailand. Oral formulation has two preparations, as fosfomycin tromethamine and as fosfomycin calcium. Both preparations are also approved by Thai FDA for uncomplicated urinary tract infection[10]. However, they are not recommended for therapy of pyelonephritis[11]. Sitafloxacin is approved by Thai FDA for urethritis, cystitis and acute pyelonephritis. Clinical trials of sitafloxacin for therapy of ESBL-producing *Escherichia coli* upper urinary tract infections are ongoing. Therefore, effective oral antibiotics for therapy of upper urinary tract infections and complicated urinary tract infections caused by ESBL-producing gram-negative bacteria are limited.

Tebipenem pivoxil is the first oral carbapenem antibiotic available for clinical use in Japan. Tebipenem shows a broad-spectrum activity against gram-positive and gram-negative bacteria including ESBL-producing gram-negative bacteria[12]. Tebipenam pivoxil is well absorbed from oral administration. It is quickly converted to tebipenem by carboxyesterase localized at the intestinal epithelial cells and then it is transferred into blood[13]. The half-life of tebipenem is one hour. Tebipenem pivoxil is mainly excreted by the kidney, 54% to 73% of a dose[13]. There are several clinical trials of tebipenem for therapy of infections including otolaryngological infections[14] and pneumonia[15]. Tebipenem is only available in Japan for otitis media, sinusitis and pneumonia in children.

The objective of the present study was to determine the in vitro activity of tebipenem against ESBL-producing *E. coli* isolated from Thai patients and the activity of serum and urine samples collected from healthy Thai men after receiving oral tebipenem pivoxil against a clinical urinary isolate of ESBL-producing *E. coli*.

Material and Method

In vitro study

Bacterial isolates

They were 100 strains of ESBL-producing *E. coli* isolated from different hospitalized-infected patients.

Susceptibility test

Minimum inhibitory concentration (MIC) of tebipenem was performed by broth micro-dilution technique using the dry plates manufactured by Eiken Chemical Co., Ltd. The inoculum of ESBL-producing *E. coli* was prepared by growth method. Bacterial inoculum was adjusted to McFarland standard No. 1, then 25 µl of the inoculum was put into 12 ml of cation-adjusted Mueller-Hinton broth and 100 µl of the mixture was inoculated into each well. The final inoculum size was approximately $5x10^4$ CFU of bacteria in each well. The inoculated plates were incubated at 35°C in ambient air for 16-20 hours. The quality control strain was *E. coli* ATCC 25922.

In vivo study

The study was approved by Siriraj Institutional Review Board and written informed consents were obtained from all subjects.

Study subjects

They were 10 healthy men aged 18 to 40 years with no history of antibiotic use within 1 week prior to the study day. All subjects received physical examinations and laboratory tests including complete blood count, urine examination, renal function and liver function tests in order to confirm that they were healthy prior to their participation in the study. Any subject with history of hypersensitivity to beta-lactams was excluded.

Study procedures (clinical part)

The study was conducted at Siriraj Clinical Research Center in January 2013. The schedules of medication and collection of blood and urine samples from each subject are depicted in Fig. 1 Each subject had 3 ml of blood sample and 5 ml of urine sample collected at 8 a.m. on the first study day. Then he received the first dose of 300 mg of tebipenem pivoxil fine granules (Meiji Seika Kaisha, Ltd.) dissolved in 200 ml of water by mouth at 8 a.m. after breakfast. Blood samples (3 ml) and urine samples (5 ml) were then recollected at 8.30 a.m., 9 a.m., and 1 p.m. He received the second dose of tebipenem pivoxil in the same amount as the first dose by mouth at 1 p.m. after lunch. Blood samples (3 ml) and urine samples (5 ml) were recollected at 1.30 p.m., 2 p.m. and 6 p.m. He received the third dose of tebipenem pivoxil in the same amount as the first dose by mouth at 6 p.m. after dinner. Blood samples (3 ml) and urine samples (5 ml) were recollected at 6.30 p.m. and 7 p.m. The subject

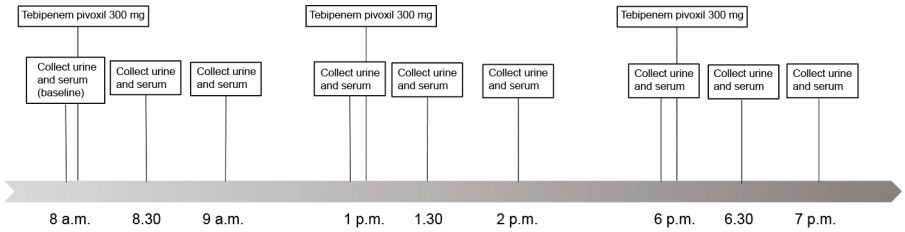

Fig. 1 The schedules of medication and collection of blood and urine samples from each subject.

stayed overnight at Siriraj Clinical Research Center. On the second study day, blood sample (3 ml) and urine sample (5 ml) were recollected at 8 a.m. Then he received the fourth dose of tebipenem pivoxil in the same amount as the first dose by mouth at 8 a.m. after breakfast. Blood samples (3 ml) and urine samples (5 ml) were recollected at 8.30 a.m., 9 a.m. and 1 p.m. He received the fifth dose of tebipenem pivoxil in the same amount as the first dose by mouth at 1 p.m. after lunch. Blood sample (3 ml) and urine sample (5 ml) were recollected at 1.30 p.m., 2 p.m. and 6 p.m. He received the sixth dose of tebipenem pivoxil in the same amount as the first dose by mouth at 6 p.m. after dinner. Blood sample (3 ml) and urine sample (5 ml) were recollected at 6.30 p.m. and 7 p.m. Then the subject left Siriraj Clinical Research Center. The collected urine samples were kept at -80°C. Serum samples were separated from the collected blood and they were kept at -80°C.

Study procedures (microbiological part)

- Study organism

The study organism was a urinary isolate of ESBL-producing *E. coli*. Its minimum inhibitory concentration (MIC) of tebipenem was ≤0.06 µg/ml. This isolate was randomly selected from 100 strains of ESBL-producing, tebipenem-susceptible *E. coli* isolates that were used in the in vitro study.

- Inhibitory activity test by disk diffusion

The study organism was prepared at the amount of 0.5 Mc Farland standard. The prepared study organism was inoculated onto the surface of Mueller-Hinton agar (MHA) plate. Then the blank disks were placed on each agar plate and 10 µl of clinical specimens (urine and serum) were dropped on each blank disk. The inoculated agar plate with clinical specimen immersed disks was then incubated at 35°C for 16-18 hours and the inhibition zone around each immersed disk was measured. These same procedures were repeated with all urine and serum samples collected from each study subject at each time point. The tebipenem 10 µg/disk was also tested and its inhibition zone diameter against the study organism was 32 mm.

- Inhibitory and bactericidal activity test by broth microdilution

Urine and serum samples were diluted into microdilution wells that contained Mueller-Hinton broth to achieve a dilution of 1:5, 1:10, 1:20, 1:40, 1:80, 1:160 and 1:320. An equal volume of 50 µl of Mueller-Hinton broth containing 106 CFU/ml of the study organism was inoculated into each microdilution well to achieve a dilution of 1:10, 1:20, 1:40, 1:80, 1:160, 1:320 and 1:640. The microdilution plate was incubated at 35°C for 16-20 hours. Then all microdilution wells were inspected for visible growth and the volume of 20 µl from each clear well was sub-cultured on brain heart infusion agar. The inhibitory activity was defined as the lowest titer of the well without visible growth. The bactericidal activity was defined as the lowest titer of the well that killed more than or equal to 99.95% of inoculated bacteria.

Data analysis

The data were analyzed by descriptive statistics. Categorical variables were reported in terms of percentage while continuous variables were expressed in terms of mean ± SD or median and range as appropriate.

Results

In vitro study

MIC_{50} and MIC_{90} of tebipenem against ESBL-producing *E. coli* were both ≤0.06 mg/L with MIC range from ≤0.06 to 0.25 mg/L.

In vivo study

Characteristics of all study subjects are shown in Table 1. All of them were adult Thai healthy men with a mean age of 30.1 years. Side effects related to

receiving tebipenem pivoxil were not experienced by any subjects.

Inhibitory activities of serum and urine samples detected by the disk diffusion method are shown in Table 2 and 3. The serum samples collected from all study subjects before receiving tebipenem pivoxil did not produce inhibition zones. The inhibition zones were observed around the disks inoculated with serum samples collected from all study subjects after receiving tebipenem pivoxil for 30 minutes and 1 hour with the median inhibition zone diameter of 15 mm and 13.5 mm, respectively. However, the inhibition zones were absent around the discs inoculated with serum samples collected from all study subjects before receiving the next dose of tebipenem pivoxil. The urine samples collected from all study subjects, except one, before receiving tebipenem pivoxil did not produce an inhibition zone. The inhibition zones were observed around the disks inoculated with urine samples collected from all subjects after receiving tebipenem pivoxil for up to 5 hours. The urine samples collected from eight subjects contained inhibitory activity of urine samples up to 14 hours after receiving tebipenem pivoxil. The median diameters of inhibition zones of urine samples were much larger than those of the serum samples.

Inhibitory and bactericidal activities of serum and urine samples detected by microdilution method are shown in Fig. 2-5 and Table 4, 5. The serum samples of the study subjects collected before receiving tebipenem pivoxil contained no or low inhibitory or bactericidal titers. The inhibitory and bactericidal titers of the serum samples collected after receiving tebipenem pivoxil were increased up to 1:320. However, the serum samples of the subjects collected before receiving the next dose of tebipenem pivoxil contained no or low inhibitory or bactericidal titers. The urine samples of the subjects collected before receiving tebipenem pivoxil contained no or low inhibitory or bactericidal titers. The inhibitory and bactericidal titers of the urine samples collected from the study subjects after receiving tebipenem pivoxil were all higher than 1:640 and lasted for at least 5 hours after the dose. The urine samples of four subjects still contained high inhibitory and bactericidal titers (≥1:160) at 14 hours after receiving tebipenem pivoxil.

Table 1. Characteristics of 10 study subjects

Characteristic	Value
Mean age, years (SD)	30.10 (5.02)
Mean body weight, Kg (SD)	65.95 (9.42)
Mean height, cm (SD)	169.30 (4.30)
Normal physical examination (%)	10 (100)
Complete blood count	
Mean hemoglobin, g/dl (SD)	13.75 (0.79)
Mean white blood cell $x10^3$/ul (SD)	7.14 (2.55)
Mean platelets $x10^3$/ul (SD)	254.20 (39.56)
Mean serum creatinine, mg/dl (SD)	0.90 (0.10)
Liver function	
Mean aspartate aminotransferase (SD)	24.60 (7.38)
Mean alanine aminotransferase (SD)	26.90 (20.20)
Mean alkaline phosphatase (SD)	67.10 (16.00)
Urinalysis proteinuria, n (%)	1 (10.0)
Glycosuria, n (%)	0 (0)

Discussion

The in vitro study results confirmed that tebipenem is very active against ESBL-producing *E. coli* isolated from Thai patients. The in vivo study was conducted in healthy men only in order to avoid having contamination with bacteria in urine during midstream urine collection as much as possible since the antibacterial activity of serum and urine samples was determined by microbiological methods, i.e. disk diffusion and broth microdilution. These methods should be more convenient and more clinically relevant to efficacy of therapy with tebipenem than measuring the levels of tebipenem in serum and urine after the subjects received tebipenem. Six subjects had low titers of serum inhibitory or bactericidal activities against ESBL-producing *E. coli* prior to the oral administration of tebipenem pivoxil. This observation should not be due to extrinsic factors, such as antibiotic use, because all subjects had no history of antibiotic use within one week prior to the study. Even if the subject did receive oral antibiotics prior to the study, it was unlikely to be able to observe such activities because ESBL-producing *E. coli* should not be susceptible to commonly used oral antibiotics, such as amoxicillin. Therefore, the observed low titers of serum inhibitory or bactericidal activity against ESBL-producing *E. coli* of these six study subjects should be due to an intrinsic factor. Tichaczek-Goska et al reported the bactericidal activity of normal human serum against enterobacteriaceae with lipopolysaccharides possessing O-antigens composed of mannan and it was postulated that such bactericidal activity of normal human serum might be mediated through complement systems[16]. One subject had inhibitory and bactericidal activity in urine samples against ESBL-producing *E. coli* detected by both disk diffusion and broth microdilution. Such

Table 2. Inhibition zone diameters of serum samples of 10 subjects against ESBL-producing *E. coli*

Subject (time)	1	2	3	4	5	6	7	8	9	10
D1 8:00 a.m.	6[1], 6[2]	6, 6	6, 6	6, 6	6, 6	6, 6	6, 6	6, 6	6, 6	6, 6
D1 8:30 a.m.	15, 14	14, 15	15, 15	13, 14	15, 15	12, 12	13, 13	13, 14	13, 14	12, 13
D1 9:00 a.m.	15, 15	13, 13	15, 16	14, 15	14, 15	12, 12	14, 15	13, 14	12, 13	13, 14
D1 1:00 p.m.	6, 6	6, 6	6, 6	6, 6	6, 6	6, 6	6, 6	6, 6	7, 7	6, 6
D1 1:30 p.m.	17, 17	16, 15	17, 17	17, 18	15, 16	14, 15	15, 15	16, 17	17, 17	14, 15
D1 2:00 p.m.	15, 15	14, 15	16, 16	14, 15	13, 14	13, 13	15, 15	14, 15	14, 15	13, 13
D1 6:00 p.m.	6, 6	6, 6	6, 6	6, 6	6, 6	6, 6	6, 6	6, 6	6, 6	6, 6
D1 6:30 p.m.	16, 17	15, 15	15, 16	13, 13	13, 14	15, 16	11, 12	17, 18	15, 16	11, 11
D1 7:00 p.m.	15, 16	14, 13	14, 15	12, 14	12, 13	12, 13	14, 15	16, 17	13, 13	12, 12
D2 8:00 a.m.	6, 6	6, 6	6, 6	6, 6	6, 6	6, 6	6, 6	6, 6	6, 6	6, 6
D2 8:30 a.m.	16, 15	16, 15	15, 16	15, 16	13, 13	12, 13	14, 14	16, 17	15, 14	12, 13
D2 9:00 a.m.	14, 15	14, 15	13, 13	15, 16	12, 13	11, 11	14, 13	14, 13	13, 13	11, 12
D2 1:00 p.m.	6, 6	6, 6	6, 6	6, 6	6, 6	6, 6	6, 6	6, 6	6, 6	6, 6
D2 1:30 p.m.	15, 16	15, 16	16, 17	15, 16	14, 15	13, 14	14, 15	16, 17	15, 15	14, 15
D2 2:00 p.m.	16, 15	13, 14	14, 14	13, 14	12, 12	12, 12	13, 12	15, 15	13, 14	14, 14
D2 6:00 p.m.	6, 6	6, 6	6, 6	6, 6	7, 8	6, 6	6, 6	6, 6	6, 6	6, 6
D2 6:30 p.m.	18, 17	16, 17	15, 16	16, 17	15, 15	13, 14	14, 15	16, 17	12, 13	12, 13
D2 7:00 p.m.	14, 15	13, 14	14, 15	14, 15	14, 14	12, 13	14, 14	14, 15	13, 12	13, 14

D = day

[1] Inhibition zone of the first disk, [2] Inhibition zone of the second disk

Table 3. Inhibition zone diameters of urine samples of 10 subjects against ESBL-producing *E. coli*

Subject (time)	1	2	3	4	5	6	7	8	9	10
D1 8:00 a.m.	6[1], 6[2]	6, 6	10, 11	6, 6	6, 6	6, 6	6, 6	6, 6	6, 6	6, 6
D1 8:30 a.m.	30, 30	32, 33	32, 33	28, 29	28, 29	31, 32	28, 29	30, 31	26, 27	31, 31
D1 9:00 a.m.	35, 35	35, 34	28, 28	31, 31	27, 28	32, 32	28, 28	31, 32	27, 28	33, 34
D1 1:00 p.m.	25, 26	30, 31	24, 23	23, 24	28, 27	25, 26	28, 29	29, 30	27, 28	24, 25
D1 1:30 p.m.	29, 30	35, 36	33, 33	27, 28	30, 31	31, 31	26, 27	32, 33	33, 34	31, 32
D1 2:00 p.m.	30, 30	34, 35	32, 33	31, 32	33, 34	29, 30	28, 28	30, 31	29, 30	29, 29
D1 6:00 p.m.	20, 21	27, 28	15, 15	24, 25	28, 29	22, 23	27, 28	28, 29	27, 28	21, 22
D1 6:30 p.m.	32, 33	34, 34	29, 30	30, 30	30, 30	30, 30	25, 26	33, 34	32, 33	28, 29
D1 7:00 p.m.	34, 35	33, 34	27, 28	29, 30	27, 27	28, 29	27, 28	32, 33	29, 30	30, 31
D2 8:00 a.m.	16, 16	29, 29	13, 13	10, 9	7, 7	6, 6	7, 6	6, 6	24, 25	10, 11
D2 8:30 a.m.	33, 34	35, 36	31, 31	33, 33	29, 30	29, 30	26, 27	31, 32	30, 31	31, 32
D2 9:00 a.m.	31, 32	32, 32	28, 29	36, 37	28, 29	30, 31	27, 27	31, 32	28, 29	34, 35
D2 1:00 p.m.	28, 29	30, 30	24, 25	27, 28	26, 27	20, 20	25, 26	29, 30	26, 27	28, 29
D2 1:30 p.m.	33, 34	34, 35	30, 30	33, 34	30, 31	28, 29	27, 28	32, 33	28, 29	30, 30
D2 2:00 p.m.	34, 34	31, 32	29, 30	31, 32	31, 32	28, 28	30, 31	29, 30	28, 29	30, 31
D2 6:00 p.m.	22, 23	30, 30	25, 26	29, 30	28, 28	24, 25	24, 25	26, 27	26, 27	22, 23
D2 6:30 p.m.	30, 31	31, 31	25, 25	32, 32	32, 32	26, 26	27, 28	31, 32	29, 30	27, 28
D2 7:00 p.m.	31, 32	34, 35	27, 28	34, 35	31, 32	26, 27	27, 27	30, 31	28, 28	27, 28

D = day

[1] Inhibition zone of the first disk, [2] Inhibition zone of the second disk

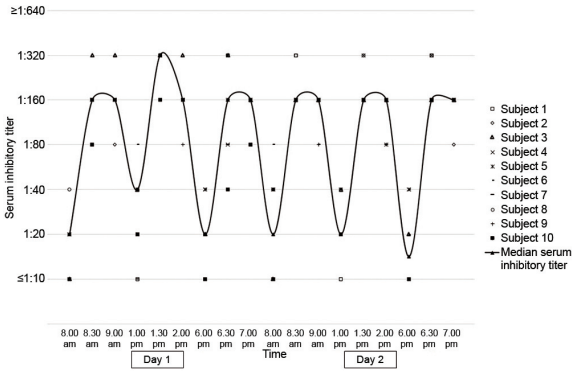

Fig. 2 Serum inhibitory titers of 10 study subjects against ESBL-producing *E. coli*.

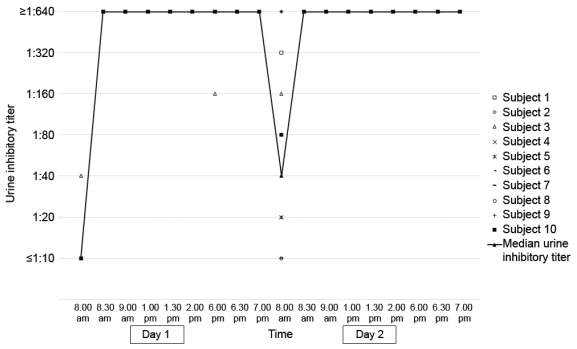

Fig. 3 Urine inhibitory titers of 10 study subjects against ESBL-producing *E. coli*.

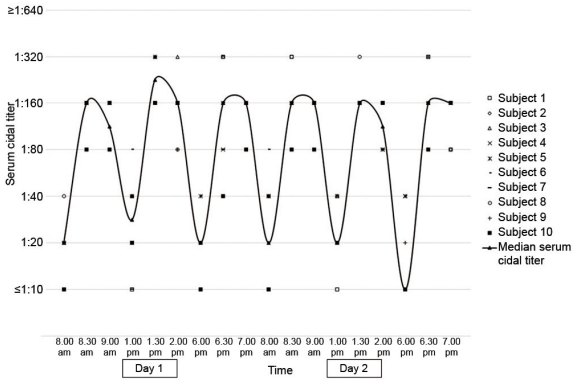

Fig. 4 Serum bactericidal titers of 10 study subjects against ESBL-producing *E. coli*.

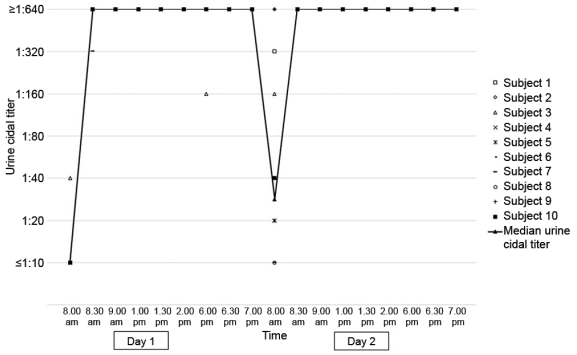

Fig. 5 Urine bactericidal titers of 10 study subjects against ESBL-producing *E. coli*.

observations should be an intrinsic activity of urine as well, although the serum samples collected from this particular subject did not exhibit inhibitory or bactericidal activity.

The high peak inhibitory and bactericidal activity in serum was observed in the samples collected at 30 minutes after receiving oral tebipenem pivoxil. These findings indicated that the absorption of tebipenem from gastrointestinal tract was good and the conversion of tebipenem pivoxil to tebipenem was rapid. However, the inhibitory and bactericidal activity in serum samples collected at 5 hours after receiving tebipenem pivoxil were not detected since the half-life of tebipenem is only one hour. Serial urine samples collected since 30 minutes after receiving oral tebipenem pivoxil contained very high inhibitory and bactericidal activity for up to 5 to 14 hours since 54% to 73% of tebipenem was excreted by the kidney[13].

The most common adverse reaction of tebipenem reported in clinical study of tebipenem pivoxil was diarrhea (≥5%). Other uncommon adverse reactions (<5%) were rash, thrombocytopenia, leukocytosis, elevated AST, ALT, blood urea nitrogen level, proteinuria, difficulty of micturition[17]. All subjects in our study who received oral tebipenem pivoxil 300 mg for 6 doses tolerated the study medication well and they did not experience any serious adverse reactions related to tebipenem pivoxil.

Our findings indicated that tebipenem pivoxil has a potential for being an oral antibiotic for therapy of outpatients with infections caused by ESBL-producing gram-negative bacteria and for being a step down therapy after parenteral carbapenem as well. This postulation should be confirmed in clinical study of therapy of patients infected with ESBL-producing gram-negative bacteria, especially upper urinary tract infections.

Conclusion

Tebipenem is very active against ESBL-producing *E. coli* isolated from Thai patients. Oral

Table 4. Inhibitory and bactericidal titers of serum samples of 10 subjects against ESBL-producing *E. coli*

Subject (time)	1		2		3		4		5		6		7		8		9		10	
	Inhib.	Cidal	Inhib.	Cidal	Inhib.	Cidal	Inhib.	Cidal	Inhib.	Cidal	Inhib.	Cidal	Inhib.	Cidal	Inhib.	Cidal	Inhib.	Cidal	Inhib.	Cidal
D1 8:00 a.m.	≤1:10	≤1:10	1:20	1:20	≤1:10	≤1:10	1:20	1:20	1:20	1:20	1:20	1:20	1:20	1:20	1:40	1:40	≤1:10	≤1:10	≤1:10	≤1:10
D1 8:30 a.m.	1:160	1:160	1:160	1:160	1:320	1:80	1:160	1:160	1:160	1:160	1:160	1:160	1:80	1:80	1:160	1:160	1:160	1:80	1:80	1:80
D1 9:00 a.m.	1:160	1:160	1:80	1:80	1:320	1:160	1:160	1:160	1:160	1:160	1:160	1:160	1:160	1:80	1:160	1:80	1:160	1:80	1:160	1:80
D1 1:00 p.m.	≤1:10	≤1:10	1:40	1:40	1:40	1:40	1:20	1:20	1:40	1:40	1:80	1:80	≤1:10	≤1:10	1:40	1:40	1:40	1:20	1:20	1:20
D1 1:30 p.m.	1:320	1:160	1:320	1:320	1:320	1:320	1:320	1:320	1:320	1:160	1:320	1:320	1:160	1:160	1:160	1:160	1:320	1:320	1:160	1:160
D1 2:00 p.m.	1:160	1:160	1:160	1:160	1:320	1:320	1:160	1:160	1:160	1:160	1:160	1:160	1:160	1:160	1:160	1:80	1:80	1:80	1:160	1:160
D1 6:00 p.m.	≤1:10	≤1:10	1:20	1:20	1:20	1:20	1:40	1:20	1:40	1:40	1:40	1:40	1:20	1:20	1:20	1:20	≤1:10	≤1:10	≤1:10	≤1:10
D1 6:30 p.m.	1:320	1:320	1:160	1:160	1:320	1:320	1:80	1:80	1:160	1:160	1:160	1:160	1:80	1:80	1:320	1:320	1:320	1:160	1:40	1:40
D1 7:00 p.m.	1:80	1:80	1:160	1:160	1:160	1:160	1:160	1:160	1:160	1:160	1:160	1:160	1:160	1:160	1:160	1:160	1:160	1:160	1:80	1:80
D2 8:00 a.m.	≤1:10	≤1:10	1:40	1:40	≤1:10	≤1:10	1:40	1:40	1:40	1:40	1:80	1:80	≤1:10	≤1:10	1:40	1:40	≤1:10	≤1:10	≤1:10	≤1:10
D2 8:30 a.m.	1:320	1:320	1:160	1:160	1:160	1:160	1:160	1:160	1:160	1:160	1:160	1:160	1:160	1:160	1:320	1:320	1:160	1:160	1:160	1:80
D2 9:00 a.m.	1:160	1:160	1:160	1:160	1:160	1:160	1:160	1:160	1:160	1:160	1:80	1:80	1:160	1:160	1:160	1:160	1:80	1:80	1:160	1:80
D2 1:00 p.m.	≤1:10	≤1:10	1:20	1:20	1:40	1:40	1:40	1:40	1:20	1:20	1:20	1:20	1:20	1:20	1:40	1:40	1:20	1:20	1:20	1:20
D2 1:30 p.m.	1:160	1:160	1:160	1:160	1:160	1:160	1:320	1:160	1:160	1:160	1:160	1:160	1:160	1:160	1:320	1:320	1:160	1:160	1:160	1:160
D2 2:00 p.m.	1:160	1:160	1:160	1:160	1:160	1:160	1:160	1:80	1:80	1:80	1:80	1:80	1:80	1:80	1:160	1:160	1:160	1:80	1:160	1:160
D2 6:00 p.m.	≤1:10	≤1:10	≤1:10	≤1:10	1:20	≤1:10	1:40	1:40	1:40	1:40	1:40	1:40	≤1:10	≤1:10	≤1:10	≤1:10	1:20	1:20	≤1:10	≤1:10
D2 6:30 p.m.	1:320	1:320	1:160	1:160	1:160	1:160	1:320	1:320	1:160	1:160	1:160	1:160	1:160	1:160	1:320	1:320	1:160	1:160	1:160	1:80
D2 7:00 p.m.	1:160	1:80	1:80	1:80	1:160	1:160	1:160	1:160	1:160	1:160	1:160	1:160	1:160	1:160	1:160	1:160	1:160	1:160	1:160	1:160

D = day; Inhib. = inhibitory titer; Cidal = bactericidal titer

Table 5. Inhibitory and bactericidal titers of urine samples of 10 subjects against ESBL-producing *E. coli*

Subject (time)	1		2		3		4		5		6		7		8		9		10	
	Inhib.	Cidal	Inhib.	Cidal	Inhib.	Cidal	Inhib.	Cidal	Inhib.	Cidal	Inhib.	Cidal	Inhib.	Cidal	Inhib.	Cidal	Inhib.	Cidal	Inhib.	Cidal
D1 8:00 a.m.	≤1:10	≤1:10	≤1:10	≤1:10	1:40	1:40	≤1:10	≤1:10	≤1:10	≤1:10	≤1:10	≤1:10	≤1:10	≤1:10	≤1:10	≤1:10	≤1:10	≤1:10	≤1:10	≤1:10
D1 8:30 a.m.	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	1:320	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640
D1 9:00 a.m.	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640
D1 1:00 p.m.	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640
D1 1:30 p.m.	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640
D1 2:00 p.m.	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640
D1 6:00 p.m.	≥1:640	≥1:640	≥1:640	≥1:640	1:160	1:160	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640
D1 6:30 p.m.	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640
D1 7:00 p.m.	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640
D2 8:00 a.m.	1:320	1:320	≥1:640	≥1:640	1:160	1:160	1:20	1:20	1:20	1:20	≤1:10	≤1:10	≤1:10	≤1:10	≤1:10	≤1:10	≥1:640	≥1:640	1:80	1:40
D2 8:30 a.m.	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640
D2 9:00 a.m.	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640
D2 1:00 p.m.	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640
D2 1:30 p.m.	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640
D2 2:00 p.m.	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640
D2 6:00 p.m.	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640
D2 6:30 p.m.	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640
D2 7:00 p.m.	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640	≥1:640

D = day; Inhib. = inhibitory titer; Cidal = bactericidal titer

administration of tebipenem pivoxil 300 mg three times a day for two consecutive days is well tolerated and safe. The serum and urine samples collected from the healthy subjects after receiving oral tebipenem pivoxil contain high inhibitory and bactericidal activity against ESBL-producing *E. coli*. Oral tebipenem pivoxil could be effective for therapy of ESBL-producing gram-negative infections, especially in upper urinary tract infection.

What is already known on this topic?

Tebipenem is used in children with respiratory tract infections in Japan.

What this study adds?

Tebipenem pivoxil is active both in vitro and in vivo against ESBL-producing *E. coli* isolated from Thai patients. Tebipenem pivoxil may be an oral agent for therapy of ESBL-producing *E. coli* infections in Thai patients.

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Potential conflicts of inferest

None.

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ฤทธิ์ของยา tebipenem ต่อเชื้อ E. coli สายพันธุ์ที่สร้าง extended spectrum beta-lactamase (ESBL) จากการทดสอบในห้องปฏิบัติการและในคน

วิษณุ ธรรมลิขิตกุล, นริสร หล่อจีระชุณห์กุล, สุรภี เทียนกริม

วัตถุประสงค์: *เพื่อทราบฤทธิ์ของยา tebipenem ต่อเชื้อ E. coli สายพันธุ์ที่สร้าง ESBL จากการทดสอบในห้องปฏิบัติการและในคน*

วัสดุและวิธีการ: *การทดสอบฤทธิ์ของยา tebipenem ต่อเชื้อ E. coli สายพันธุ์ที่สร้าง ESBL ในห้องปฏิบัติการ ทำโดยหาค่า minimum inhibitory concentration (MIC) ด้วยวิธี broth microdilution ส่วนการทดสอบฤทธิ์ของยา tebipenem ต่อเชื้อ E. coli สายพันธุ์ที่สร้าง ESBL ในคนทำในอาสาสมัครชายจำนวน 10 คน โดยทดสอบฤทธิ์ของซีรัมและปัสสาวะของอาสาสมัครภายหลังกินยา tebipenem pivoxil ขนาด 300 มิลลิกรัม วันละ 3 ครั้ง ติดต่อกัน 2 วัน ด้วยวิธี disk diffusion และ broth microdilution*

ผลการศึกษา: *ค่า MIC_{50} และ MIC_{90} ของยา tebipenem ต่อเชื้อ E. coli สายพันธุ์ที่สร้าง ESBL คือ ≤0.06 มิลลิกรัม/ลิตร ทั้งสองค่า โดยมีพิสัยตั้งแต่ ≤0.06 ถึง 0.25 มิลลิกรัม/ลิตร ซีรัมและปัสสาวะของอาสาสมัครภายหลังกินยา tebipenem pivoxil ทำให้เกิด inhibition zone รอบ disk อย่างน้อย 1 ชั่วโมง และ 5 ชั่วโมง ตามลำดับ ซีรัมของอาสาสมัครภายหลังกินยา tebipenem pivoxil มีฤทธิ์ยับยั้งและฤทธิ์ทำลายเชื้อที่ระดับ 1 ต่อ 160 นานอย่างน้อย 1 ชั่วโมง ปัสสาวะของอาสาสมัครภายหลังกินยา tebipenem pivoxil มีฤทธิ์ยับยั้งและฤทธิ์ทำลายเชื้อที่ระดับ 1 ต่อ 640 นานอย่างน้อย 14 ชั่วโมง อาสาสมัครทุกรายไม่มีผลข้างเคียงจากการกินยา tebipenem pivoxil*

สรุป: *Tebipenem มีฤทธิ์ดีมากต่อเชื้อ E. coli สายพันธุ์ที่สร้าง ESBL การกินยา tebipenem pivoxil ขนาด 300 มิลลิกรัม วันละ 3 ครั้ง ติดต่อกัน 2 วัน มีความปลอดภัย และทำให้ซีรัมและปัสสาวะของอาสาสมัครภายหลังกินยา tebipenem pivoxil มีฤทธิ์ยับยั้ง และฤทธิ์ทำลายเชื้อ E. coli สายพันธุ์ที่สร้าง ESBL ยา tebipenem pivoxil น่าจะเป็นยากินที่มีประสิทธิผลในการรักษาการติดเชื้อ E. coli สายพันธุ์ที่สร้าง ESBL*