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J Infect Chemother (2010) 16:280–287
DOI 10.1007/s10156-010-0053-9

ORIGINAL ARTICLE

Investigation of pneumonia-causing pathogenic organisms in children and the usefulness of tebipenem pivoxil for their treatment

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Received: 13 October 2009 / Accepted: 19 February 2010 / Published online: 31 March 2010

Abstract We investigated the usefulness of the novel oral carbapenem antibiotic tebipenem pivoxil (TBPM-PI) for treating bacterial pneumonia in children. Sputum and nasopharyngeal swabs were collected simultaneously, and causative organisms were identified by conventional bacterial culture together with exhaustive bacterial and viral identification by real-time polymerase chain reaction (PCR). The subjects were eight patients diagnosed with mild or moderate pneumonia at Sotobo Children's Clinic Outpatient Department between October 2006 and June 2007. TBPM-PI was administered at the recommended clinical dose of 4 mg/kg b.i.d. to five patients and at a high dose of 6 mg/kg b.i.d. to three patients. Sputum was collected from all patients, and 11 strains were detected from washed sputum culture. Causative organisms were mainly *Streptococcus pneumoniae* (3 strains) and *Haemophilus influenzae* (6 strains), and nasopharyngeal swabs showed the same organisms as coughed-up sputum. Real-time PCR for individual viruses and *Mycoplasma pneumoniae* identified four cases of only bacterial infection, one case of *M. pneumoniae* coinfection, two cases of viral coinfection, and one case of both viral and *M. pneumoniae* coinfection. The clinical results indicated efficacy in all patients, and causative organisms were 100% eliminated. In the four patients with only bacterial infection, the average fever of 38.9°C at the start of treatment normalized the following day, showing excellent efficacy. No clinically problematic adverse events occurred, and compliance was good. We consider that these cases provide valuable insights into the identity of pathogenic organisms of pneumonia in children and the possible role of TBPM-PI in outpatient treatment.

Keywords Tebipenem pivoxil · Oral carbapenem · Pneumonia · Child · Sputum · PCR

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Introduction

Pneumonia in children is a disease that is seen relatively frequently in general practice. To identify the causative organisms, it is important to collect sputum and conduct washed sputum culture. Actually, however, washed sputum culture is seldom conducted in the primary care setting [1, 2]. It is considered that the clinical condition of pneumonia is essentially an infection caused by diverse pathogenic organisms such as *Mycoplasma pneumoniae* and various viruses in addition to pathogenic bacteria [3]. Tests that can detect these diverse organisms in the sputum of children are also seldom performed.

In principle, inpatient hospital care is essential for moderate and more severe cases of pneumonia [4], but hospitalization of a child places a large burden on both the family and the child. It is necessary to improve outpatient treatment for the disease.

Here, we studied the efficacy of the novel oral carbapenem antibiotic tebipenem pivoxil (TBPM-PI) for treating bacterial pneumonia in children [5]. For bacteriological tests, sputum and nasopharyngeal swabs were collected simultaneously, and causative organisms were identified by conventional bacterial culture in parallel with exhaustive bacterial and viral identification by real-time polymerase chain reaction (PCR) [5]. This report summarizes cases where washed sputum culture was performed at our clinic as part of a clinical trial. We consider that these cases

provide valuable insights into the identity of pathogenic organisms of pneumonia in children and the possibility of outpatient treatment of these patients.

The examination obtained the approval of the clinical test screening committee, and I observed ethical principles based on the Helsinki Declaration, the 28th public welfare departmental order "departmental order (GCP) about the standard of the enforcement of the clinical test of medical supplies" dated March 27, 1997, and a clinical test enforcement plan that I carried out.

Subjects and methods

Target cases

The study was conducted in patients with mild or moderate pneumonia who were seen at the outpatient department of Sotobo Children's Clinic, Japan, during the period of October 2006–June 2007. Pneumonia presumably caused by general bacteria was targeted. The severity of the pneumonia was judged in reference to "Evaluation criteria for clinical trials of antimicrobial drugs in the pediatric field" [6] of the Japanese Society of Chemotherapy.

Inclusion criteria were patients who met all three conditions of (1) clinical symptoms of respiratory infection such as fever and cough, (2) chest X-ray findings of pneumonia, and (3) white blood cell count (WBC) $\geq$10,000/μl, or C-reactive protein (CRP) $\geq$2 mg/dl. Age was set as at least 6 months and less than 16 years, and body weight as at least 7 kg and less than 50 kg.

Patients with a severe infectious disease or severe complication, patients who were not suitable for clinical evaluation, those having convulsive disorders such as epilepsy, and patients having native carnitine deficiency disease were excluded from the subjects.

Patients' consent

Before a patient was entered into the clinical study, the study was sufficiently explained to the patient's legal guardian, and the legal guardian gave written consent by his/her own free will for the patient to participate in the clinical trial.

Administration and dosage

In principle, TBPM-PI was administered at 4 mg (potency)/kg of body weight at a time, twice a day (b.i.d.) after a meal. Depending on the symptoms and severity, however, 6 mg/kg b.i.d. was administered. The administration period was 7 days (or a total of 8 administration days). The dose was not changed during the administration period.

Exclusion criteria and other details are as previously reported [5].

Observations, tests, and investigated items

Observations and tests were performed before treatment, on day 3 of treatment, and at the end of treatment or on discontinuation. The final observations were performed 7–14 days after the end of treatment (hereinafter, final observation day).

Patient profile

Before each patient was started on this clinical study, the following items were recorded: sex, birth date, height, body weight, in- or outpatient status, diagnosis and severity of the infection, normal body temperature, underlying diseases or complications, allergies, past history, and concomitant drugs or therapies.

Clinical symptoms or findings

Before treatment, on day 3 of treatment, at the end of treatment or on discontinuation, and on the final observation day, the following items were observed or investigated: body temperature, cough, respiratory rate, sputum, respiratory distress (forced breathing), chest pain, symptoms of dehydration, cyanosis, crepitations, and lethargy.

Laboratory tests

Before treatment and at the end of treatment or on discontinuation, the following items were measured: CRP (quantitative), WBC count, differential WBC count, red blood cell (RBC) count, hemoglobin, hematocrit, platelet count, aspartate aminotransferase (AST), alanine transaminase (ALT), gamma-glutamyl transpeptidase (γ-GTP), total bilirubin (T-bil), blood urea nitrogen (BUN), serum creatinine, Na, K, and Cl, Urinary protein, urinary glucose, and urinary urobilinogen were measured only for patients in whom it was possible. On day 3 of treatment and on the final observation day, the foregoing tests, excluding urinary protein, urinary glucose, and urinary urobilinogen, were conducted as necessary.

Bacteriological tests

Before treatment, on day 3 of treatment, at the end of treatment or on discontinuation, and, in principle, on the final observation day, sputum and nasopharyngeal swabs were collected. The methods for sputum collection and

washed culture are as previously reported [2, 7]. Identification of the bacterial strains isolated from the specimens, measurement of the bacterial content, drug sensitivity measurement by the agar plate dilution method, bacterial and viral identification by real-time PCR [8, 9], as well as drug-susceptibility genotyping of *Streptococcus pneumoniae* and *Haemophilus influenzae* [10, 11], and biological typing tests [12, 13] by real-time PCR were conducted at the Kitasato Institute for Life Sciences.

Chest X-rays

Before treatment and at the end of treatment or on discontinuation, a chest X-ray was taken. The pneumonia score was evaluated based on the guidelines for calculating the chest X-ray shadow score in the "Clinical evaluation method of novel antimicrobial agents for respiratory infections (draft)" [14] of the Japanese Society of Chemotherapy.

Method of evaluating efficacy

Clinical efficacy

Judgment of the clinical efficacy Based on the "Evaluation criteria for clinical trials of antimicrobial drugs in the pediatric field" [6] of the Japanese Society of Chemotherapy, changes in the main clinical symptoms and findings were used to classify the clinical efficacy into the four grades of "excellent," "good," "fair," and "poor," or "impossible to judge."

Judgment at the final observation If clinical efficacy was confirmed at the end of treatment, and it was considered unnecessary to additionally administer antimicrobial agents or prohibited concomitant drugs based on the clinical symptoms and findings, until the final observation day or thereafter, the disease was judged as "cured." If it was necessary to administer a new antimicrobial drug for the disease, it was judged as "not cured." If neither of these judgments could be made, the disease was "impossible to judge."

Bacteriological efficacy

The causative organisms were evaluated based on the properties of the specimen, according to the Geckler classification of bacteria that were isolated from the washed sputum. The presence of the causative organisms at the end of treatment or on discontinuation was judged as either, "became negative," "continue to exist," or "impossible to judge." If the clinical symptoms improved, and the sputum disappeared after TBPM-PI treatment, it was handled as "became negative." The elimination rate was set as the percentage of bacterial strains that were judged as "became negative."

Safety and compliance

Safety and compliance were evaluated as previously reported [5].

Results

Composition of cases

Breakdown of cases

The number of target cases was eight. In all eight cases, the pathogenic organisms were investigated, and the efficacy and safety of, and compliance with, TBPM-PI treatment were evaluated.

Administration and dosage was 4 mg/kg b.i.d. in five cases and 6 mg/kg b.i.d. in three cases.

I used the dosage 6 mg/kg b.i.d. for cases with more than 3 points of the pneumonia score, more than CRP 4 mg/dl, more than 10,000 WBC count/μl, and temperature more than 38°C.

Patient profile

Patient profile by type of infection The results of treatment in the eight patients to whom TBPM-PI was administered, the patient profile by type of infection, and the symptoms and findings before the start of treatment are shown in Tables 1 and 2. Cases no. 2 and 3 had previously received claritromycin (CAM). *M. pneumoniae* was isolated from case no. 2. Case no. 8, from whom influenza A virus was isolated, did not receive antivirus treatment.

Resistance distribution of causative organisms The breakdown of causative organisms that were isolated from washed sputum were 3 strains of *S. pneumoniae*, 6 strains of *H. influenzae*, and 2 strains of *Streptococcus pyogenes*. *S. pneumoniae* and *H. influenzae* were classified by the minimum inhibitory concentration (MIC) set by the Clinical and Laboratory Standards Institute (CLSI) [15] and by genotype based on the mechanism of resistance analyzed by real-time PCR.

All 3 strains of *S. pneumoniae* were resistant (I + R) to benzylpenicillin (PCG), and consisted of genotypic penicillin-resistant *S. pneumoniae* (gPRSP) with amino acid substitution of the penicillin-binding proteins (PBPs) 1a, 2x, and 2b, which contribute to PCG resistance. All 3 strains were also clarithromycin (CAM)-resistant strains (R) that possessed ermB or mefA. Among the 6 *H. influenzae* strains, 3 strains were ampicillin (ABPC) resistant

Table 1 Clinical summary of eight infected patients treated with tebipenem pivoxil (TBPM-PI)

Case No.	Age (sex)	Clinical diagnosis (severity)	Underlying disease	Previous antimicrobial treatment	Daily dose (days)	Organism isolated from washed sputum Before → After	Chest X-P (score)	Evaluation		Adverse reactions	
								Bacteriological	Clinical	Symptoms	Lab findings
1	4 years 3 months (F)	Pneumonia (moderate)	Asthmatic bronchitis	–	6 mg/kg × 2 (8)	*S. pneumoniae* *S. pyogenes* ↓ No sputum	Improved (3 → 1)	Eradicated	Excellent	–	–
2	5 years 0 month (F)	Pneumonia (moderate)	–	CAM	6 mg/kg × 2 (7)	*H. influenzae* *M. pneumoniae* ↓ No sputum	Improved (4 → 2)	Eradicated	Excellent	–	–
3	4 years 1 month (M)	Pneumonia (moderate)	Asthmatic bronchitis	CAM	4 mg/kg × 2 (7)	*H. influenzae* ↓ No sputum	Improved (4 → 1)	Eradicated	Excellent	–	–
4	0 years 8 months (M)	Pneumonia (moderate)	Asthmatic bronchitis Conjunctivitis Diarrheal disease	–	6 mg/kg × 2 (7)	*H. influenzae* *M. pneumoniae* rhino virus ↓ No sputum	Improved (7 → 2)	Eradicated	Good	Stools watery	–
5	5 years 1 month (M)	Pneumonia (moderate)	–	–	4 mg/kg × 2 (7)	*S. pneumoniae* *H. influenzae* ↓ No sputum	Improved (7 → 2)	Eradicated	Excellent	Mushy stool	PLT↑ (24.7 → 72.8)
6	2 years 2 months (F)	Pneumonia (moderate)	Diarrheal disease	–	4 mg/kg × 2 (7)	*S. pneumoniae* *H. influenzae* ↓ No sputum	Improved (2 → 1)	Eradicated	Excellent	–	–
7	0 year 8 months (F)	Pneumonia (mild)	Diarrheal disease	–	4 mg/kg × 2 (8)	Normal flora corona virus ↓ No sputum	Improved (7 → 2)	Unknown	Good	Mushy stool Erythema	–
8	3 years 6 months (F)	Pneumonia (mild)	Constipation Dry eczema Asthmatic bronchitis	–	4 mg/kg × 2 (8)	*H. influenzae* *S. pyogenes* influenza A virus ↓ No sputum	No change (2 → 3)	Eradicated	Good	–	–

Table 2 Patient profiles

Item	Patients by type of infection				
	Pathogenic bacteria ($n = 4$)	*M. pneumoniae* + pathogenic bacteria ($n = 1$)	Viral + pathogenic bacteria ($n = 2$)	*M. pneumoniae* + Viral + pathogenic bacteria ($n = 1$)	Total ($n = 8$)
Gender					
Male	2	0	0	1	3
Female	2	1	2	0	5
Age (years)					
≥3 to <6	3	1	1	0	5
≥0.5 to <3	1	0	1	1	3
Body weight (kg)					
≥7 to <10	0	0	1	1	2
≥10 to <20	4	1	1	0	6
Category					
Inpatient	0	0	0	0	0
Outpatient	4	1	2	1	8
Severity of infection					
Mild	0	0	2	0	2
Moderate	4	1	0	1	6
Medical history					
No	4	1	2	1	8
Yes	0	0	0	0	0
Underlying disease and/or complication					
No	1	1	0	0	2
Yes	3	0	2	1	6
Previous antimicrobial treatment					
No	3	0	1	2	6
Yes	1	1	0	0	2
Dose					
4 mg/kg bid	3	0	2	0	5
6 mg/kg bid	1	1	0	1	3

(I + R): the 3 strains were gBLNAR (β-lactamase non-producing ampicillin-resistant *H. influenzae*) and gBLPAR (β-lactamase-positive ampicillin-resistant *H. influenzae*), which have an amino acid substitution caused by a variant *ftsI* gene, which contributes to ABPC resistance.

Susceptibility distribution of causative organisms For *S. pneumoniae*, the MIC of PCG ranged from 1 to 2 μg/ml, and all 3 strains showed penicillin resistance and low susceptibility to cephem and macrolide antimicrobials. The MIC of TBPM, however, ranged from 0.031 to 0.063 μg/ml, showing excellent susceptibility. For *H. influenzae*, the MIC of ABPC ranged from 0.25 to 8 μg/m, whereas the MIC of TBPM ranged from 0.063 to 0.5 μg/ml.

Comparison of the microorganisms isolated from washed sputum and nasopharyngeal swabs The results of comparisons of the microorganisms isolated from sputum and from nasopharyngeal swabs taken before the start of treatment are shown for each case in Table 3.

Microorganisms isolated from the sputum were basically the same as those isolated from nasopharyngeal swabs. In particular, organisms that were detected in the sputum and considered to be the causative organisms were also detected in nasopharyngeal swabs. Genotype and capsular serotype were also identical.

The results of individual viral and *M. pneumoniae* identifications by real-time PCR showed that four cases had coinfection with viruses and *M. pneumoniae*, and these results were also basically the same for the sputum and the nasopharyngeal swabs.

Evaluation of efficacy

Clinical efficacy

For the four cases with only bacterial infection, and the one case of coinfection with *M. pneumoniae*, five cases in total, clinical efficacy was judged to be "excellent." For the two

Table 3 Isolated organisms from washed sputum and nasopharyngeal swabs for each case

Case no.	Washed sputum		Nasopharyngeal swabs	
	Organism isolated (culture)	Resistance type/sero type (PCR)	Isolated organism (culture)	Resistant type/sero type (PCR)
1	[a]*S. pneumoniae* (+++)	gPRSP (pbp1a + pbp2x + pbp2b), ermB/23F	*S. pneumoniae*	gPRSP (pbp1a + pbp2x + pbp2b), ermB/23F
	[a]*S. pyogenes* (+++)		*S. pyogenes*	gLow-BLNAR
			H. influenzae	
2	[a]*H. influenzae* (+++)	gBLNAR	*H. influenzae*	gBLNAR
	S. aureus	MSSA	α-Streptococcus	
	α-Streptococcus	*M. pneumoniae* (30 cycles)		
3	[a]*H. influenzae* (++)	gBLNAS	*H. influenzae*	gBLNAS
			H. influenzae	gBLPACR-II, type b
4	[a]*H. influenzae* (+++)	gBLNAR	*H. influenzae*	gBLNAR
		M. pneumoniae (32 cycles) rhino virus (++)		rhino virus
5	[a]*S. pneumoniae* (++)	gPRSP (pbp1a + pbp2x + pbp2b), ermB/23F	*S. pneumoniae*	gPRSP (pbp1a + pbp2x + pbp2b), ermB/23F
	[a]*H. influenzae* (+++)	gBLPAR, type b	*H. influenzae*	gBLPAR, type b
6	[a]*H. influenzae* (+)	gLow-BLNAR	*H. influenzae-S. pneumoniae*	gLow-BLNAR
	[a]*S. pneumoniae* (+)	gPRSP (pbp1a + pbp2x + pbp2b), mefA/19F		gPRSP (pbp1a + pbp2x + pbp2b), mefA/19F
7	*M. catarrhalis*	MSSA	Normal flora	
	S. aureus	corona virus (+++)		corona virus
	S. agalactiae			
8	[a]*H. influenzae* (++)	gBLNAS	*H. influenzae-S. pyogenes*	gBLNAS
	[a]*S. pyogenes* (++)	influenza A virus (+++)		influenza A virus

[a] Causative organisms

cases with viral coinfection and the one case with viral and *M. pneumoniae* coinfection, three cases in total, it was judged to be "good:" thus, the efficacy was 100% (8/8 cases). The final observation was judged as "cured" for all cases. In addition, the difference was not excepted by a clinical response according to the dose.

Bacteriological efficacy

Concerning the bacteriological efficacy at the end of treatment or on discontinuation, the 3 strains of *S. pneumoniae*, 6 strains of *H. influenzae*, and 2 strains of *S. pyogenes* all "became negative" independently of the type of infection, and the elimination rate was 100% (11/11 strains). "Became negative" was judged based on the disappearance of sputum in association with improvement in all clinical symptoms.

Investigation of safety and compliance

The approved side effects were watery stools (one), mushy stools (two), erythema (one), and number of the blood platelets increased in examination (one). All side effects were resolved immediately after the dosage was completed. The case in which examination was canceled by side effect expression was not accepted.

There were seven cases judged as "Very easy to take" and "Easy to take", there was one case of an 8-month-old baby judged as "Common" and there was no case judged as "Hard to take" and "Unable to take."

Discussion

We investigated the causative organisms isolated from the sputum from cases of pneumonia in children, and the efficacy of TBPM-PI for the treatment of pneumonia in children.

Pathogens such as *H. influenzae*, *S. pneumoniae*, and *Moraxella catarrhalis* were detected in the washed sputum and were the same as the causative organisms that have been reported previously [16]. This investigation was characterized by the isolation of other pathogenic organisms from the sputum by means of PCR. *M. pneumoniae,* rhinovirus, coronavirus, and influenza virus were isolated

from the sputum. Lower respiratory infections in children are not necessarily caused by a single bacterium, and coinfection with viruses is also found [17]. As previously reported, it was also shown that coinfection of *M. pneumoniae* with bacteria is not at all rare [18]. The causative organisms that were isolated from washed sputum culture were considered to be highly infectious compared with those isolated from nonwashed sputum, the nasopharynx, or the throat [17]. In this study, in parallel with washed sputum culture, various organisms were also detected in sputum by using PCR. This test is considered particularly valuable considering the pathology of lower respiratory infections.

In this study, culture of nasopharyngeal swab fluid was also conducted in parallel with PCR. Basically, the same results as those for the washed sputum cultures were found. It is considered useful to collect nasopharyngeal swab fluid from patients in whom the collection of sputum is impossible; but sputum and washed sputum cultures are in principle superior for identifying the pathogens of lower respiratory disease.

The detected causative bacteria are 3 strains of *S. pneumoniae*, 6 strains of *H. influenzae*, and 2 strains of *S. pyogenes*, which are basically the same strains as those previously reported [1, 16]. In this study, viral and *M. pneumoniae* identification was performed by real-time PCR. The results showed that among the eight cases, four cases had only bacterial infection, and four cases had viral and/or *M. pneumoniae* coinfection. Two of the cases with viral coinfection had mild disease (Table 1; cases 7 and 8).

For all four cases with only bacterial infection, the fever, which averaged 38.9°C at the start of treatment, normalized the following day, and clinical efficacy was excellent. The three patients in whom the clinical efficacy was good (cases nos. 4, 7, and 8) all had viral coinfection with or without *M. pneumoniae*. The fever took longer to subside in these cases compared with the cases that showed excellent efficacy; this is considered to be the result of the additional healing processes for the viral and *M. pneumoniae* coinfections in children. The patient in whom only a small amount of *M. pneumoniae* was isolated by PCR (case no. 2) was the one that had been previously treated with CAM, and the role of *M. pneumoniae* in the present case of pneumonia was considered to be rather small.

In recent years, penicillin resistance of *S. pneumoniae* and *H. influenzae* has been increasing, and in this study too, among the eight cases, two were infected with resistant *S. pneumoniae*, two with resistant *H. influenzae*, and one with both. In all these cases of pneumonia involving resistant bacteria, TBPM-PI showed superior clinical efficacy judged as "good" or better.

At present, the "Guidelines for the Management of Respiratory Infectious Diseases in Children" [4] handles the diagnosis and treatment of pneumonia by age and severity. According to the severity criteria in the "Evaluation criteria for clinical trials of antimicrobial drugs in the pediatric field" [6] of the Japanese Society of Chemotherapy, the eight cases in this study had mild or moderate disease; but according to the severity criteria in the "Guidelines for the Management of Respiratory Infectious Diseases in Children," they were all evaluated to have severe disease for which hospitalization is recommended. TBPM-PI was shown to be effective in such cases for which hospitalization is recommended. In particular, for the four cases that had only bacterial infections, the average fever, which was 38.9°C at the start of treatment, normalized the following day, showing rapid clinical efficacy. Until now the treatment of such cases of pneumonia was generally considered to depend on hospitalization of the child to administer intravenous antimicrobials. We can treat some patients with pneumonia of moderate degree with oral cephems and high-dose penicillin. The sensitivity of oral cephems to *S. pneumoniae* decreased recently, and some children have difficulty in taking high-dose oral penicillin. Thus, we have the superiority of TBPM-PI for the treatment of mild to moderate pneumonia in outpatients. We should administer TBPM-PI carefully, with consideration of its cost and the development of resistant bacteria. The treatment with oral cephems or high-dose penicillin is sometimes not efficacious for pneumonia caused by some resistant organisms, such as penicillin-resistant *S. pneumoniae* and ampicillin-resistant *H. influenzae*. We administer TBPM-PI as a second choice of antimicrobial agents for pneumonia in children in cases in which oral cephems or high-dose penicillin is not effective. Appropriate use of carbapenem is important for preventing resistant bacteria.

Hospitalization places a huge psychological and economic burden on the guardians, in addition to the effect on the child. TBPM-PI will make it possible to treat such cases of pneumonia, which previously have needed hospitalization, on an outpatient basis by combining its use with oral rehydration therapy, thus lightening the psychological and economic burden.

Acknowledgments We thank Prof. Kimiko Ubukata of the Laboratory of Molecular Epidemiology for Infectious Agents, Graduate School of Infection Control Sciences, Kitasato Institute, for her cooperation with the identification of organisms isolated from specimens, the measurement of their content and drug susceptibility, identification of bacteria and individual viruses by real-time PCR, as well as drug-resistance genotyping of *Streptococcus pneumoniae* and *Haemophilus influenzae* and biological typing by real-time PCR during the conduct of this study.

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