International Journal of Antimicrobial Agents 38 (2011) 70–75

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: http://www.elsevier.com/locate/ijantimicag

Short communication

In vitro activities of ceftobiprole combined with amikacin or levofloxacin against *Pseudomonas aeruginosa*: evidence of a synergistic effect using time–kill methodology

Michael Kresken[a,*], Barbara Körber-Irrgang[a], Jörg Läuffer[b], Sabine Decker-Burgard[c], Todd Davies[d]

[a] *Antiinfectives Intelligence GmbH, Campus of the University of Applied Sciences Bonn-Rhein-Sieg, Von-Liebig-Straße 20, 53359 Rheinbach, Germany*
[b] *Janssen EMEA, Baar, Switzerland*
[c] *Janssen GmbH, Neuss, Germany*
[d] *Johnson & Johnson Pharmaceutical Research & Development, L.L.C., Raritan, NJ, USA*

ARTICLE INFO

Article history:
Received 1 November 2010
Accepted 25 January 2011

Keywords:
Ceftobiprole
Pseudomonas aeruginosa
Kill curve
Synergism
Combination

ABSTRACT

Ceftobiprole is an investigational intravenous broad-spectrum cephalosporin with in vitro activity against Gram-positive and Gram-negative pathogens, including meticillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa* is a frequent nosocomial pathogen, increasingly associated with complicated skin and skin-structure infections. Combination antimicrobial therapy is recommended as empirical therapy for serious infections where *P. aeruginosa* is suspected. Therefore, in this study the interaction of ceftobiprole with two other antipseudomonal agents (amikacin and levofloxacin) was investigated. Time–kill studies were performed for each single agent and for the combination of ceftobiprole 4 mg/L with either amikacin or levofloxacin at 0.5×, 1× and 2× the minimum inhibitory concentration. Five clinical isolates of *P. aeruginosa* as well as the *P. aeruginosa* ATCC 27853 reference strain were tested at initial inocula of 5×10^5 colony-forming units (CFU)/mL (low inoculum) or 5×10^7 CFU/mL (high inoculum). Synergy was defined as a decrease of $\geq 2 \log_{10}$ CFU/mL with the combination compared with the most active single drug at 6 h and 24 h. At low inoculum with ceftobiprole as a single agent, viable counts were decreased by 1.5–2 $\log_{10}$ at 6 h. Addition of either amikacin or levofloxacin resulted in synergistic bactericidal activity at 24 h. At high inoculum the combination of ceftobiprole with amikacin or levofloxacin demonstrated synergism in one of three and three of five strains, respectively. This study demonstrated that the combination of ceftobiprole at a clinically achievable concentration of 4 mg/L with amikacin or levofloxacin exhibited synergistic activity against *P. aeruginosa*. There was no evidence of antagonism for either combination.

1. Introduction

Pseudomonas aeruginosa is a highly drug-resistant and opportunistic pathogen [1]. It has become a leading cause of Gram-negative infection both in hospitals and the community and it has been reported as one of the principal causes of nosocomial infection, particularly amongst immunocompromised patients [1]. Although Gram-positive organisms, including *Staphylococcus aureus*, are the most common pathogens in skin infections, Gram-negative bacilli, and particularly *P. aeruginosa*, are also recovered from complicated infections [2].

Guidelines recommend combination therapy with two drugs to cover *P. aeruginosa* for treating patients with serious infections or when drug-resistant organisms are suspected [3]. Empirical initial combination therapy is superior to monotherapy for *P. aeruginosa*-associated bacteraemia and ventilator-associated pneumonia [4]. Combination therapy, frequently given empirically for severe *P. aeruginosa*, usually comprises an adequate β-lactam and an aminoglycoside or fluoroquinolone [4].

Ceftobiprole is a broad-spectrum investigational cephalosporin with activity against Gram-positive and Gram-negative pathogens, including clinical isolates of meticillin-resistant *S. aureus* (MRSA), drug-resistant pneumococci, Enterobacteriaceae and *P. aeruginosa* [2,5]. The in vitro activity of ceftobiprole, together with clinical data, suggest that it may be useful as empirical monotherapy for complicated skin and skin-structure infections (cSSSIs) and pneumonia, in combination with other antimicrobials if additional coverage is needed in lower respiratory tract infections [6]. For the treatment of patients with cSSSI, two phase III studies have shown that ceftobiprole is as effective as vancomycin in treating Gram-positive

* Corresponding author. Tel.: +49 2226 908 912; fax: +49 2226 908 918.
E-mail address: michael.kresken@antiinfectives-intelligence.de (M. Kresken).

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doi:10.1016/j.ijantimicag.2011.01.028

infections and as effective as vancomycin plus ceftazidime in treating infections due to Gram-positive and Gram-negative bacteria [7,8].

Although there are data demonstrating synergistic activity between cephalosporins and both aminoglycosides and fluoroquinolones against *P. aeruginosa* [9–11], evidence of synergism involving ceftobiprole in the same setting is, to our knowledge, non-existent. A single study has shown synergism of ceftobiprole and aminoglycosides against *Enterococcus faecalis* [12]. The objective of this study was to evaluate the interaction of ceftobiprole with amikacin or levofloxacin against *P. aeruginosa* using time–kill methodology. Here we demonstrate that ceftobiprole at clinically achievable levels exhibits synergistic activity against *P. aeruginosa* when combined with amikacin or levofloxacin.

2. Materials and methods

2.1. Bacterial strains

Five clinical isolates of *P. aeruginosa* as well as the *P. aeruginosa* ATCC 27853 reference strain were evaluated. Strains were selected based on minimum inhibitory concentration (MIC) results to provide a range of susceptibilities to the different antimicrobial agents tested.

2.2. Antibiotics

Ceftobiprole was provided by Johnson & Johnson Pharmaceutical Research & Development, L.L.C. (Raritan, NJ). Levofloxacin was provided by Sanofi-Aventis Deutschland GmbH (Berlin, Germany). Amikacin was purchased from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany).

2.3. Susceptibility testing

MICs were determined by the broth microdilution method with geometric two-fold serial dilutions in cation-adjusted Mueller–Hinton broth (CA-MHB) (Becton Dickinson, Heidelberg, Germany) according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI). Broth microdilution studies were performed for individual antibiotics. Interpretive criteria applied to amikacin and levofloxacin were those published by the CLSI.

2.4. Time–kill assays

Time–kill assays were performed in glass flasks containing 20 mL of CA-MHB, either with each compound alone or with ceftobiprole in combination with amikacin or levofloxacin at starting inocula of ca. 5×10^5 colony-forming units (CFU)/mL (low inoculum) or 5×10^7 CFU/mL (high inoculum). The antibacterial activity of ceftobiprole was measured at 4 mg/L to simulate free drug concentrations that were previously achieved for 40–50% of an 8-h dosing interval when ceftobiprole was administered at 500 mg intravenously over 2 h to 150 subjects enrolled in phase I/II trials. The same dosing regimen was selected for a phase III trial comparing ceftobiprole with vancomycin plus ceftazidime for the treatment of patients with cSSSI caused by Gram-positive and Gram-negative pathogens [7]. Amikacin and levofloxacin were tested at concentrations corresponding to the MIC, one dilution below the MIC (0.5× MIC) and one dilution above the MIC (2× MIC) for each organism. *Pseudomonas aeruginosa* test organisms were incubated at 35 °C with vigorous shaking over 24 h. The combination of ceftobiprole and amikacin was tested against two clinical isolates (PEG-01-14 and PEG-02-54) as well as the reference strain. The combination of ceftobiprole and levofloxacin was tested against four clinical isolates (PEG-01-14, PEG-02-33, P-03-29 and P-04-03) as well as the reference strain.

Table 1
Minimum inhibitory concentrations (MICs) of ceftobiprole, amikacin and levofloxacin against *Pseudomonas aeruginosa* test strains.

P. aeruginosa strain	MIC (mg/L)		
	Ceftobiprole	Amikacin[a]	Levofloxacin[b]
ATCC 27853	2	4 (S)	2 (S)
PEG-01-14	4	4 (S)	0.5 (S)
PEG-02-33	2	N/D	4 (I)
PEG-02-54	2	8 (S)	N/D
P-03-29	8	N/D	4 (I)
P-04-03	4	N/D	1 (S)

S, susceptible; I, intermediate; N/D, not determined; CLSI, Clinical and Laboratory Standards Institute.
[a] Interpretive criteria based on CLSI recommendations: S, ≤16 mg/L; I, 32 mg/L; and R, ≥64 mg/L.
[b] Interpretive criteria based on CLSI recommendations: S, ≤2 mg/L; I, 4 mg/L; and R, ≥8 mg/L.

Briefly, 200 μL samples were removed from each culture flask at 0, 1, 2, 3, 6 and 24 h after inoculation and were added to 1.8 mL of saline. These suspensions were then serially diluted using 10-fold dilutions, with 50 μL aliquots subsequently plated onto tryptic soy agar plates (Fluka, Buchs, Switzerland) and incubated for 24 h at 37 °C. Plates showing growth of 10–100 CFU were counted and time–kill curves were plotted as $\log_{10}$ CFU/mL over time. The limit of detection was 1.3 $\log_{10}$ CFU/mL (or 2×10^1 CFU/mL). Antibiotic carryover was controlled by dilution. All experiments were performed at least in duplicate with results presented as the mean of all experiments. In each experiment, one flask containing inoculated CA-MHB without an antimicrobial agent served as a growth control.

A bactericidal effect was defined as a ≥3 $\log_{10}$ CFU/mL decrease in the viable count both at 6 h and 24 h compared with the starting inoculum. Synergism was defined as a decrease in viable count of ≥2 $\log_{10}$ CFU/mL with the combination compared with the most active single drug after 6 h and 24 h. Antagonism was defined as an increase in viable counts of ≥2 $\log_{10}$ CFU/mL with the combination compared with the most active single drug.

3. Results

3.1. Minimum inhibitory concentrations

MICs of the study drugs for the five clinical *P. aeruginosa* isolates and the *P. aeruginosa* reference strain ATCC 27853 are shown in Table 1. None of the organisms was resistant to either amikacin or levofloxacin based on CLSI interpretive criteria [13], but two isolates (PEG-02-33 and P-03-29) demonstrated intermediate susceptibility to levofloxacin. MICs for ceftobiprole ranged from 2 mg/L to 8 mg/L.

3.2. Time–kill studies

The findings of time–kill studies for each of the six *P. aeruginosa* strains during exposure to single-agent ceftobiprole 4 mg/L or to amikacin or levofloxacin (0.5×, 1× and 2× MIC) are summarised in Table 2i. At low inoculum with ceftobiprole at 4 mg/L, viable *P. aeruginosa* counts were decreased by 1.5–2 $\log_{10}$ at 6 h, with an increase in viable counts relative to the initial inoculum subsequently observed for all six strains at 24 h. With high inoculum, ceftobiprole at 4 mg/L exhibited bacteriostatic activity at 6 h, but re-growth was seen with all strains at 24 h.

Amikacin and levofloxacin both exhibited a concentration-dependent effect when tested as single agents at concentrations of 0.5×, 1× and 2× MIC, as expected. At 2× MIC, bactericidal activ-