ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 2005, p. 4372–4374 Vol. 49, No. 10
0066-4804/05/$08.00+0 doi:10.1128/AAC.49.10.4372–4374.2005

Comparative In Vitro Activity of Ceftobiprole against Staphylococci Displaying Normal and Small-Colony Variant Phenotypes

Christof von Eiff,[1]* Alexander W. Friedrich,[2] Karsten Becker,[1] and Georg Peters[1]

Institute of Medical Microbiology[1] and Institute of Hygiene,[2] University of Münster Hospital and Clinics, 48149 Münster, Germany

Received 20 May 2005/Returned for modification 19 July 2005/Accepted 26 July 2005

The antistaphylococcal activity of ceftobiprole was compared with those of cefuroxime, linezolid, and moxifloxacin by using the agar dilution method. Apart from three strains with small-colony variant phenotypes, all *Staphylococcus aureus* isolates tested were inhibited by ≤2 μg/ml of ceftobiprole. This compound exhibited an excellent antistaphylococcal activity, comparable to that of linezolid.

Ceftobiprole (BPR) (formerly BAL9141) is a novel parenteral cephalosporin with activity against a broad range of pathogens, particularly against gram-positive bacteria (3, 5, 7). While the compound has been described to possess strong affinity for the *mecA* product PBP 2a, data on the activity of ceftobiprole against well-characterized staphylococci are limited. Therefore, aims of this study were (i) to challenge the ceftobiprole spectrum by evaluating its activity against well-defined staphylococcal strains of different species, including small-colony variants (SCVs), and (ii) to compare the in vitro antistaphylococcal activity of this cephalosporin with those of linezolid, moxifloxacin, and cefuroxime.

The 284 *Staphylococcus aureus* strains tested comprised 72 methicillin-susceptible *S. aureus* (MSSA) and 212 methicillin-resistant *S. aureus* (MRSA) strains, including 14 strains with stable SCV phenotypes. The 80 coagulase-negative staphylococci (CoNS) comprised 14 methicillin-susceptible and 21 methicillin-resistant *Staphylococcus epidermidis* strains, 17 methicillin-susceptible and 15 methicillin-resistant *Staphylococcus haemolyticus* strains, and 13 other CoNS belonging to eight different species.

All staphylococcal strains were freshly isolated from clinical material and were included in the testing only if they were considered etiologically relevant. Apart from isolates with SCV phenotypes, only one isolate per patient was tested. Eighty-one MRSA strains, each presenting a different *spa* type, were selected from our institutional collection (between 1996 and 2004). All other *spa*-typed MRSA isolates were collected during the course of a multicenter study in Germany also including community-acquired MRSA, with not more than five strains selected from each center. Overall, the MRSA strains tested represent more than 90 *spa* types and thus cover >90% of all registered European MRSA *spa* types within the SeqNet network (www.SeqNet.org) (6).

If the biochemical identification of staphylococcal isolates (ATB32 Staph; bioMerieux, Marcy l'Etoile, France) was ambiguous or categorized as unacceptable, 16S rRNA gene sequencing was performed as previously described (2). Isolates

TABLE 1. Antimicrobial activities of ceftobiprole and selected comparison drugs tested against staphylococci

Antimicrobial agent against indicated organism (no. of strains tested)[a]	MIC (μg/ml)[b]		
	Range	50%	90%
MSSA (60)			
Ceftobiprole	0.13–0.5	0.25	0.5
Cefuroxime	0.25–2	2	2
Linezolid	0.5–2	1	2
Moxifloxacin	≤0.03–4	≤0.03	0.06
MRSA (197)			
Ceftobiprole	0.25–2	1	2
Cefuroxime	4–64	16	>128
Linezolid	0.25–1	1	1
Moxifloxacin	≤0.03–8	2	4
MSSE (14)			
Ceftobiprole	0.13–1	0.25	0.5
Cefuroxime	0.25–4	0.5	1
Linezolid	0.5–1	0.5	1
Moxifloxacin	≤0.03–4	0.06	2
MRSE (21)			
Ceftobiprole	0.5–4	1	2
Cefuroxime	0.5–>128	8	>128
Linezolid	0.5–1	0.5	1
Moxifloxacin	≤0.03–4	1	2
MSSH (17)			
Ceftobiprole	0.13–1	0.25	0.5
Cefuroxime	0.5–4	2	2
Linezolid	0.5–1	0.5	1
Moxifloxacin	≤0.03–4	≤0.03	2
MRSH (15)			
Ceftobiprole	1–4	2	4
Cefuroxime	8–>128	64	>128
Linezolid	0.5–1	0.5	1
Moxifloxacin	1–8	4	8
CoNS (13)			
Ceftobiprole	≤0.03–1	0.5	1
Cefuroxime	0.25–8	2	4
Linezolid	0.5–2	1	1
Moxifloxacin	≤0.03–0.25	0.13	0.25

[a] Strains exhibiting normal morphotype (results for SCVs and their parent strains are given in Table 2). MSSE, methicillin-susceptible *S. epidermidis*; MRSE, methicillin-resistant *S. epidermidis*; MSSH, methicillin-susceptible *S. haemolyticus*; MRSH, methicillin-resistant *S. haemolyticus.*

[b] Quality control of all MIC determinations was performed by using the following reference strains: *S. aureus* subsp. *aureus* ATCC 25923, ATCC 29213, and ATCC 43300, *S. epidermidis* DSM 20044, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 35218, and *Pseudomonas aeruginosa* ATCC 27853. The MICs for these strains were within acceptable limits throughout testing.

* Corresponding author. Mailing address: Institute of Medical Microbiology, University of Münster Hospital and Clinics, Domagkstraβe 10, 48149 Münster, Germany. Phone: 49-251-835-5360. Fax: 49-251-835-5350. E-mail: eiffc@uni-muenster.de.

TABLE 2. Antimicrobial activities of ceftobiprole and selected comparison drugs tested against strain pairs of small-colony variants and their clonally identical parent strains with normal morphotype

PS/SCV strain pair (resistance type)[a]	MIC (μg/ml)							
	Ceftobiprole		Cefuroxime		Linezolid		Moxifloxacin	
	PS	SCV	PS	SCV	PS	SCV	PS	SCV
01 (MRSA)	1	2	64	>128	0.5	2	2	2
02 (MRSA)	1	4	>128	>128	0.5	2	2	8
03 (MRSA)	0.5	0.5	4	4	1	1	1	2
04 (MRSA)	2	4	>128	>128	0.5	4	4	8
05 (MRSA)	0.5	0.5	4	4	0.5	2	2	2
06 (MRSA)	0.5	1	4	2	1	1	1	2
07 (MRSA)	0.5	2	4	>128	1	4	2	8
08 (MRSA)	NA[b]	4	NA	>128	NA	2	NA	8
09 (MSSA)	0.13	0.13	1	1	1	4	<0.03	<0.03
10 (MSSA)	0.5	0.13	2	1	1	4	<0.03	0.06
11 (MSSA)	0.25	0.25	2	1	1	2	<0.03	0.13
12 (MSSA)	0.25	0.25	2	1	1	2	<0.03	0.13
13 (MSSA)	0.25	0.25	1	1	0.5	0.5	<0.03	0.06
14 (PSSA)	0.25	0.25	2	2	1	0.5	0.06	0.13

[a] PS, parent strain; PSSA, penicillin-susceptible *S. aureus*.
[b] NA, not available.

were confirmed to be methicillin resistant by supplementation of the agar with 2% NaCl (read after incubation for 48 h at 30°C by using 5-μg oxacillin disks) and by detection of the *mecA* gene (10).

S. aureus SCVs ($n = 14$) were collected from patients with persistent and/or recurrent infections, such as chronic osteomyelitis or chronic skin and soft tissue infections (11–13). Isolates were recognized as SCVs and genotyped as previously described (11–13). Strains with SCV phenotypes were confirmed as *S. aureus* by testing for the *S. aureus*-specific *nuc* gene (9). For comparison, 13 isolates with normal morphotypes (clonally identical to the corresponding SCVs), which were recovered in the same or subsequent clinical specimens as the SCVs, were also included in this study.

The MICs were determined by using the agar dilution technique according to CLSI (4). Ceftobiprole, cefuroxime, linezolid, and moxifloxacin were obtained from their respective manufacturers. The test range was 0.03 to 128 μg/ml (up to 32 μg/ml for ceftobiprole). The results were read after 18 h of incubation at 36°C, and the results for SCVs and their parent strains were read also after 42 h and 66 h of incubation. Several reference strains were included as controls. Additionally, sterility and growth controls were always performed.

The MIC distribution data for the *S. aureus* strains with normal phenotypes as well as for the CoNS are shown in Table 1. Ceftobiprole exhibited excellent wide-spectrum antistaphylococcal activity. According to the MIC at which 50% of isolates were inhibited (MIC_{50}) and MIC_{90} values, ceftobiprole was two- to eightfold more active than cefuroxime against methicillin-susceptible staphylococci, encompassing *S. aureus* and 10 different species of CoNS. While all *S. aureus* isolates with normal morphotypes, including MRSA isolates covering >90% of *spa* types registered in Europe, were inhibited by ≤2 μg/ml of ceftobiprole, cefuroxime MIC_{90}s for methicillin-resistant staphylococci (MRS) were all greater than 128 μg/ml. By comparison, activity of ceftobiprole was similar to linezolid against MRSA; however, ceftobiprole was slightly more active than the oxazolidinone against methicillin-susceptible strains. While moxifloxacin was the most active agent against MSSA strains, the in vitro activity of the fluoroquinolone against the MRS panel was poor.

The MICs of the agents tested against stable SCVs and their isogenic parent strains with normal morphotypes are shown in Table 2. While the number of SCV isolates tested is limited, it should be stressed that eight different clonal lineages of MRSA with SCV phenotypes and six different clonal lineages of MSSA with SCV phenotypes were included in this study (9). Of interest, antimicrobials were often less active against SCVs tested than their isogenic parent strains, with consistently low MICs for ceftobiprole. This phenomenon has been clearly described for aminoglycosides but has not been demonstrated for cephalosporins, fluroroquinolones, or linezolid (1). Nevertheless, apart from three strains (MICs, 4 μg/ml), all *S. aureus* isolates with SCV phenotypes, including those exhibiting methicillin resistance, were inhibited by ≤2 μg/ml of ceftobiprole.

MRS, including MRSA isolates with SCV phenotypes, present a major challenge in terms of chemotherapy because effective antimicrobial treatment options for infections caused by these isolates are close to becoming exhausted. In this context, the ability of ceftobiprole to inhibit staphylococci with a methicillin resistance phenotype may be of major clinical importance. Except for three strains with SCV phenotypes, all *S. aureus* strains tested, including a large number of different *spa* types, were inhibited by ≤2 μg/ml of ceftobiprole. In fact, the antistaphylococcal activity of ceftobiprole was comparable to that of linezolid, the cephalosporin being even more active against methicillin-susceptible strains than the oxazolidinone. In previous studies testing the activity of ceftobiprole against staphylococci, smaller numbers of resistance phenotypes were tested to challenge the ceftobiprole spectrum and isolates from the same patient were not excluded (5, 7, 8). Of particular importance, multiple isolates of the same strain were not excluded by phenotypic or genomic typing. In contrast, we tested a large number of clonally unrelated strains, which is particularly important for MRSA. Furthermore, a broad spectrum of

different, well-characterized staphylococcal species recovered from patients with overt infections, including isolates with SCV phenotypes, were encompassed in the testing. Thus, the range of MICs of antimicrobials documented in this study was broader than those observed in previous studies (7, 8, 14).

Overall, in our study, the newly developed agent ceftobiprole was highly active against both unrelated methicillin-susceptible and -resistant staphylococci, stimulating further evaluation of this agent for therapy of staphylococcal infections.

We sincerely thank S. Weber and A. Terliesner for expert technical assistance.

REFERENCES

1. **Baumert, N., C. von Eiff, F. Schaaff, G. Peters, R. A. Proctor, and H. G. Sahl.** 2002. Physiology and antibiotic susceptibility of *Staphylococcus aureus* small colony variants. Microb. Drug Resist. **8:**253–260.
2. **Becker, K., D. Harmsen, A. Mellmann, C. Meier, P. Schumann, G. Peters, and C. von Eiff.** 2004. Development and evaluation of a quality-controlled ribosomal sequence database for 16S ribosomal DNA-based identification of *Staphylococcus* species. J. Clin. Microbiol. **42:**4988–4995.
3. **Chambers, H. F.** 2005. Evaluation of ceftobiprole in a rabbit model of aortic valve endocarditis due to methicillin-resistant and vancomycin-intermediate *Staphylococcus aureus*. Antimicrob. Agents Chemother. **49:**884–888.
4. **CLSI (formerly NCCLS).** 2005. Performance standards for antimicrobial susceptibility testing; 15th informational supplement (M100–S15). CLSI, Wayne, Pa.
5. **Deshpande, L., P. R. Rhomberg, T. R. Fritsche, H. S. Sader, and R. N. Jones.** 2004. Bactericidal activity of BAL9141, a novel parenteral cephalosporin against contemporary gram-positive and gram-negative isolates. Diagn. Microbiol. Infect. Dis. **50:**73–75.
6. **Harmsen, D., H. Claus, W. Witte, J. Rothganger, H. Claus, D. Turnwald, and U. Vogel.** 2003. Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for *spa* repeat determination and database management. J. Clin. Microbiol. **41:**5442–5448.
7. **Hebeisen, P., I. Heinze-Krauss, P. Angehrn, P. Hohl, M. G. Page, and R. L. Then.** 2001. In vitro and in vivo properties of Ro 63-9141, a novel broad-spectrum cephalosporin with activity against methicillin-resistant staphylococci. Antimicrob. Agents Chemother. **45:**825–836.
8. **Jones, R. N., L. M. Deshpande, A. H. Mutnick, and D. J. Biedenbach.** 2002. In vitro evaluation of BAL9141, a novel parenteral cephalosporin active against oxacillin-resistant staphylococci. J. Antimicrob. Chemother. **50:**915–932.
9. **Kipp, F., K. Becker, G. Peters, and C. von Eiff.** 2004. Evaluation of different methods to detect methicillin resistance in small-colony variants of *Staphylococcus aureus*. J. Clin. Microbiol. **42:**1277–1279.
10. **Murakami, K., W. Minamide, K. Wada, E. Nakamura, H. Teraoka, and S. Watanabe.** 1991. Identification of methicillin-resistant strains of staphylococci by polymerase chain reaction. J. Clin. Microbiol. **29:**2240–2244.
11. **Proctor, R. A., D. M. Bates, and P. J. McNamara.** 2001. Electron transport-deficient *Staphylococcus aureus* small-colony variants as emerging pathogens, p. 95–110. *In* W. M. Scheld, W. A. Craig, and J. M. Hughes (ed.), Emerging infections 5. ASM Press, Washington, D.C.
12. **von Eiff, C., K. Becker, D. Metze, G. Lubritz, J. Hockmann, T. Schwarz, and G. Peters.** 2001. Intracellular persistence of *Staphylococcus aureus* small-colony variants within keratinocytes: a cause for antibiotic treatment failure in a patient with Darier's disease. Clin. Infect. Dis. **32:**1643–1647.
13. **von Eiff, C., D. Bettin, R. A. Proctor, B. Rolauffs, N. Lindner, W. Winkelmann, and G. Peters.** 1997. Recovery of small colony variants of *Staphylococcus aureus* following gentamicin bead placement for osteomyelitis. Clin. Infect. Dis. **25:**1250–1251.
14. **von Eiff, C., and G. Peters.** 1999. Comparative in-vitro activities of moxifloxacin, trovafloxacin, quinupristin/dalfopristin and linezolid against staphylococci. J. Antimicrob. Chemother. **43:**569–573.