It must be noted that NO in SNP also has a nitrosonium (NO^+) character[9] and is capable of reacting with cysteine residues according to:

$$[Fe^{II}(CN)_5NO^+]^{2-} + RS^- \leftrightarrow [Fe(CN)_5N(O)SR]^{3-} \quad (2)$$

followed by the release of free NO and the formation of a dimer (RSSR) according to:

$$[Fe^{II}(CN)_5N(O)SR]^{3-} + H_2O \rightarrow [Fe^{II}(CN)_5H_2O]^{3-} + NO + 1/2\ RSSR \quad (3)$$

However, these reactions are very slow at a physiological pH, where the thiol groups are mainly protonated. Evidently, the direct exchange of NO from SNP by a proton of an SH group is impossible in such a coordination complex. Therefore, SNP can be considered unable to promote the direct inactivation of a cysteine residue through a transnitrosation reaction and this characteristic may be the cause for its non-significant amoebicidal action. It is, thus, possible that GSNO and SNAC may react directly with *Acanthamoeba* cysteine proteases, like caspases, leading to their effective inactivation. Of course, other molecular targets can also be involved in the actions of GSNO and SNAC.

These results suggest that *S*-nitrosothiols are potential therapeutic drugs for the treatment of *Acanthamoeba* infections. Further studies aimed at understanding the molecular mechanisms involved in the amoebicidal actions of GSNO and SNAC, their pharmacokinetic and pharmacodynamic parameters, and their ocular toxicity in animal models of *Acanthamoeba* keratitis are necessary.

Funding

This work was supported in part by 'Fundação de Amparo à Pesquisa do Estado de São Paulo' (FAPESP; 04/00819-0 to G. F. P. de S.).

Transparency declarations

None to declare.

References

1 Clarke DW, Niederkorn JY. The pathophysiology of *Acanthamoeba* keratitis. *Trends Parasitol* 2006; **22**: 175–80.

2 Sun X, Zhang Y, Li R *et al*. *Acanthamoeba* keratitis: clinical characteristics and management. *Ophthalmology* 2006; **113**: 412–6.

3 Turner NA, Russell AD, Furr JR *et al*. Emergence of resistance to biocides during differentiation of *Acanthamoeba castellanii*. *J Antimicrob Chemother* 2000; **46**: 27–34.

4 Hogg N. The biochemistry and physiology of *S*-nitrosothiols. *Annu Rev Pharmacol Toxicol* 2002; **42**: 585–600.

5 de Souza GFP, Yokoyama-Yasunaka JK, Seabra AB *et al*. Leishmanicidal activity of primary *S*-nitrosothiols against *Leishmania major* and *Leishmania amazonensis*: implications for the treatment of cutaneous leishmaniasis. *Nitric Oxide* 2006; **15**: 209–16.

6 Ricardo KFS, Shishido SM, de Oliveira MG *et al*. Characterization of the hypotensive effect of *S*-nitroso-*N*-acetylcysteine in normotensive and hypertensive conscious rats. *Nitric Oxide* 2002; **7**: 57–66.

7 de Oliveira MG, Langley GJ, Rest AJ. Photolysis of the [Fe(CN)(5)(NO)](2) ion in water and poly(vinyl alcohol) films – evidence for cyano radical, cyanide ion and nitric oxide loss and redox pathways. *J Chem Soc Dalton Trans* 1995; **12**: 2013–9.

8 de Oliveira MG, Shishido SM, Seabra AB *et al*. Thermal stability of primary *S*-nitrosothiols: roles of autocatalysis and structural effects on the rate of nitric oxide release. *J Phys Chem A* 2002; **106**: 8963–70.

9 Gaston B, Reilly J, Drazen JM *et al*. Endogenous nitrogen oxides and bronchodilator *S*-nitrosothiols in human airways. *Proc Natl Acad Sci USA* 1993; **90**: 10957–61.

10 Sajid M, McKerrow JH. Cysteine proteases of parasitic organisms. *Mol Biochem Parasitol* 2002; **120**: 1–21.

J Antimicrob Chemother 2010
doi:10.1093/jac/dkp483
Advance publication 18 January 2010

In vitro bactericidal activity of ceftobiprole against hospital- and community-associated methicillin-resistant *Staphylococcus aureus*

Sonia Borbone, Floriana Campanile, Dafne Bongiorno and Stefania Stefani*

Department of Microbiology, University of Catania (I), Via Androne 81, 95124 Catania, Italy

*Corresponding author. Tel: +39-095-2504714; Fax: +39-095-2504734; E-mail: stefanis@unict.it

Keywords: MRSA, CA-MRSA, HA-MRSA, hVISA, killing curves, paradoxical killing, population analysis

Sir,

Ceftobiprole, formerly designated BAL9141/Ro63-9141, is a pyrrolidinone-3-ylidene-methyl cephalosporin with activity against methicillin-resistant *Staphylococcus aureus* (MRSA), Enterobacteriaceae and *Pseudomonas aeruginosa*. This anti-MRSA characteristic represents a remarkable evolution of the cephalosporin class of antimicrobial agents that have good coverage of Gram-negative bacteria but have hitherto lacked activity against MRSA.[1,2] The efficacy of ceftobiprole was assessed in clinical trials of treatment of complicated skin and skin structure infections (cSSSIs) and it was recently approved in Canada for this indication including non-limb-threatening diabetic foot infections without osteomyelitis.[3] Further Phase III clinical trials of cSSSIs have recently been completed and are under review by the US FDA and the European Medicines Agency.

The objective of this investigation was to evaluate the antibacterial and bactericidal activity of ceftobiprole, compared with those of other drugs, against a group of clinically relevant and molecularly characterized healthcare-associated (HA) and community-associated (CA) MRSA strains isolated in Italy. The strains are representative of MRSA currently circulating in hospitals and the community in Italy.[4]

One hundred HA-MRSA and 16 CA-MRSA, isolated during 2007–8, were molecularly characterized by PFGE, staphylococcal cassette chromosome (SCC)*mec* and multilocus sequence typing (MLST) (http://mlst.zoo.ox.ac.uk) methods, and the presence of the *pvl* gene was detected, following protocols previously published.[4,5] *In vitro* susceptibility testing for ceftobiprole (Johnson & Johnson Pharmaceutical Research & Development, Raritan, NJ, USA) and other major anti-Gram-positive drugs was performed by the broth microdilution method to determine MICs, following CLSI guidelines.[6] Ceftobiprole was prepared by the addition of 99 μL of DMSO and 10 μL of glacial acetic acid to 1.5 mg of powder and then diluted with 891 μL of distilled water. MBCs, as well as time–kill experiments (1×, 2× and 4× the MIC), were performed only for ceftobiprole, using standard procedures.[7,8] Sixteen strains were randomly selected for time–kill analysis, and experiments were performed in triplicate using a standard inoculum of $\sim 10^6$ cfu/mL. The lowest limit of detection for colony counts was 2 $\log_{10}$ cfu/mL and bactericidal activity was defined as a $\geq$3 $\log_{10}$ cfu/mL (99.9%) reduction from the starting inoculum. Population analysis was performed as previously reported,[9] and adapted for ceftobiprole.

The overall MIC_{90} value for ceftobiprole was 2 mg/L, the MICs ranging from 0.5 mg/L [for sequence type (ST) 22 and CA-MRSA] to 4 mg/L (ST247); the MIC_{90} for the multidrug-resistant (MDR) hVISA (heteroresistant vancomycin-intermediate *S. aureus*) strains was 4 mg/L. HA- or CA-MRSA were fully susceptible to daptomycin (MIC_{90} 1 mg/L for all HA-MRSA strains including hVISA and one dilution less for the CA-MRSA isolates), linezolid (MIC_{90} of 2 mg/L) and tigecycline (the same MIC_{90} for all HA-MRSA including hVISA and one dilution less for the CA-MRSA strains) (Table 1). Following the recent EUCAST (European Committee on Antibiotic Susceptibility Testing) clinical MIC breakpoints revised in September 2009,[10] 18% of HA-MRSA strains were resistant to teicoplanin, while all CA-MRSA strains

Table 1. Cumulative susceptibility results for 100 HA-MRSA and 16 CA-MRSA

								No. of isolates with the indicated MIC (mg/L)							
Drugs	Strain type	ST-SCC*mec*	PFGE	No.	MIC range (mg/L)	MIC_{50} (mg/L)	MIC_{90}/MBC_{90}	0.06	0.12	0.25	0.5	1	2	4	8
CBP	all strains			116	0.25–4	1	2/4			1	33	30	26	26	
	HA-MRSA	ST8-I	A1	8	0.5–1	0.5	1/1				4	4			
	HA-MRSA	ST247-IA	A2	16	1–4	2	4/4					2	10	4	
	HA-MRSA	ST247-I/IA	C	12	1–4	4	4/4					4		8	
	HA-MRSA	ST239-IIIA	B	8	1–2	2	2/4					2	6		
	HA-MRSA	ST228-I	E	24	2–4	4	4/4						10	14	
	HA-MRSA	ST22-IV	G	32	0.5–1	0.5	1/1				18	14			
	hVISA	ST8/247/239/228		12	1–4	4	4/4					2	1	9	
	CA-MRSA	ST5/8/30/80-IV ST88-V		16	0.25–1	0.5	0.5/2			1	11	4			
VAN	HA-MRSA			100	0.5–2	1	2				16	34	50		
	CA-MRSA			16	1–2	1	2	9				9	2		
TEC	HA-MRSA			100	0.06–8	0.5	4	2	4	6	40	14	16	16	2
	CA-MRSA			16	0.5–2	1	1				5	8	3		
DAP	HA-MRSA			100	0.12–1	0.5	1		4	22	28	46			
	hVISA			12	0.25–1	0.5	1			1	6	5			
	CA-MRSA			16	0.12–1	0.25	0.5		2	9	3	2			
LZD	HA-MRSA			100	0.5–4	2	4				12	34	34	20	
	hVISA			12	0.5–4	1	2				1	5	5	1	
	CA-MRSA			16	0.25–4	1	2			1	2	6	6	1	
Q/D	HA-MRSA			100	0.06–2	0.5	1	2		10	70	14	4		
	hVISA			12	0.25–1	0.5	1			2	8	2			
	CA-MRSA			16	0.25–4	1	2			3	2	5	4	2	
TGC	HA-MRSA			100	0.06–1	0.25	0.5	2	8	44	44	2			
	hVISA			12	0.12–5	0.5	0.5		2	2	8				
	CA-MRSA			16	0.12–0.5	0.25	0.25		5	9	2				

CBP, ceftobiprole; VAN, vancomycin; TEC, teicoplanin; DAP, daptomycin; LZD, linezolid; Q/D, quinupristin/dalfopristin; TGC, tigecycline.
MBCs were only determined for ceftobiprole.
hVISA strains emerged from HA-MRSA clones.

were in the susceptible range. Six of the 16 CA-MRSA strains possess a reduced susceptibility to quinupristin/dalfopristin, following the EUCAST guidelines,[10] and they showed a correlation with higher linezolid MICs, but still in the susceptible range.

Ceftobiprole was bactericidal at 1× or 2× the MIC, against all strains tested. It should be noted that a paradoxical killing effect was observed in 90% of strains. Briefly, after an initial killing (represented by the MBC values described above) the population underwent increased growth at higher concentrations of the β-lactam, followed by further killing. Population analyses performed on a representative sample of these strains failed to demonstrate the presence of heteroresistant subpopulations induced by increased concentrations of ceftobiprole.

Table 2 shows the time–kill curves performed on 16 strains; only results using ceftobiprole at 1× MIC are reported as all results obtained at other MICs were similar. The bactericidal activity of ceftobiprole was evident at 3 h against the CA-MRSA tested, at 6 h against ST8-HA-MRSA-I and at 8 h against ST228-HA-MRSA-I; bactericidal activity was obtained after 8 h also against ST22-HA-MRSA-IV. The drug was bacteriostatic (detection limit of 2 $\log_{10}$ cfu/mL) against the MDR (ST247, ST228, ST239) strains, including the hVISA strains, after 8 h.

The bactericidal and bacteriostatic behaviour was previously observed in target attainment studies for staphylococci, when free drug concentrations exceed the MIC for 30% and 50% of the dosing interval, respectively.[11] Ceftobiprole did not show a 99.9% kill activity against the hVISA strains, but was potently bactericidal against the CA-MRSA isolates. These *in vitro* data confirm the results obtained in clinical studies in cSSSI treatment, in which the drug demonstrated a higher cure (93.1%) than vancomycin (84.6%) in these strains.[12]

The paradoxical bactericidal result that we observed was not confirmed by the population analyses results. Although unclear at present, we exclude the possibility that this phenomenon could be due to: (i) heteroresistant subpopulations growing in the presence of increased concentrations of the drug; (ii) tolerance; or (iii) instability of the drug. One possible explanation could be related to a specific strain characteristic, in which an impaired autolytic function[13] could be hypothesized.

Table 2. Killing activity of ceftobiprole at the MIC

		Average log change in viable count at the MIC after			
Clones	MIC/MBC	2 h	4 h	6 h	8 h
ST8-HA-MRSA-I	0.5/1	−1.4	−2.3	−3.0	−3.6
ST247-HA-MRSA-IA	2/2	+0.7	−0.1	−0.4	−2.0
ST247-HA-MRSA-I/IA	1/2	−0.5	−1.0	−0.7	−2.0
ST239-HA-MRSA-IIIA	4/4	+0.3	−0.1	−0.7	−2.0
ST228-HA-MRSA-I	4/4	−0.7	−1.2	−2.0	−3.0
ST22-HA-MRSA-IV	0.5/1	−1.2	−1.3	−2.0	−3.2
ST8-CA-MRSA-IV	0.5/2	−1.2	−3.0	−6.0	−6.0
ST247/228-hVISA-I	4/4	−0.3	−0.8	−0.8	−2.0

Lowest limit of detection = 2 $\log_{10}$ cfu/mL.

In conclusion, ceftobiprole is a potent antistaphylococcal drug, active against CA- and HA-MRSA including hVISA strains. The bases of its antimicrobial properties, i.e. PBP2a inhibition and its stability against penicillinases, together with its broad-spectrum activity against other Gram-positive and Gram-negative bacteria, may permit its use as a single drug in human infections in which this antibiotic is efficacious.

Acknowledgements

This work was partially presented at the Nineteenth European Congress of Clinical Microbiology and Infectious Diseases, Helsinki, 2009 (Abstract P1097).

We are indebted to Dr Annalisa Pantosti and Dr Edoardo Carretto for contributing the CA-MRSA strains. We also thank Antony Bridgewood for the revision of the English of the manuscript. Ceftobiprole was kindly provided by Johnson & Johnson Pharmaceutical Research and Development, Raritan, NJ, USA.

Funding

This work was partially supported by the Italian Ministry of Education, Universities and Research (MIUR)–National Research Projects PRIN (2007SCA9RK) entitled 'An integrated view of resistance and virulence determinants in pathogenic bacteria that circulate in Italian hospitals and long term care facilities'.

Transparency declarations

None to declare.

References

1 Hebeisen P, Heinze-Krauss I, Angehrn P *et al*. In vitro and in vivo properties of Ro 63-9141, a novel broad-spectrum cephalosporin with activity against methicillin-resistant staphylococci. *Antimicrobial Agents Chemother* 2001; **45**: 825–36.

2 Vidaillac C, Rybak MJ. Ceftobiprole: first cephalosporin with activity against methicillin-resistant *Staphylococcus aureus*. *Pharmacotherapy* 2009; **29**: 511–25.

3 Noel GJ, Strauss RS, Amsler K *et al*. Results of a double-blind, randomized trial of ceftobiprole treatment of complicated skin and skin structure infections caused by Gram-positive bacteria. *Antimicrob Agents Chemother* 2008; **52**: 37–44.

4 Campanile F, Bongiorno D, Borbone S *et al*. Hospital-associated methicillin-resistant *Staphylococcus aureus* (HA-MRSA) in Italy. *Ann Clin Microbiol Antimicrob* 2009; **8**: 22.

5 Stefani S, Bongiorno D, Cafiso V *et al*. Pathotype and susceptibility profile of a community-acquired methicillin-resistant *Staphylococcus aureus* strain responsible for a case of severe pneumonia. *Diagn Microbiol Infect Dis* 2009; **63**: 100–4.

6 Clinical and Laboratory Standards Institute. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, 8th edn. Approved Standard M7–A8*. CLSI, Wayne, PA, USA, 2009.

7 Vaudaux P, Gjinovci A, Bento M *et al*. Intensive therapy with ceftobiprole medocaril of experimental foreign-body infection by methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2005; **49**: 3789–93.

8 Leonard SN, Cheung CM, Rybak MJ. Activities of ceftobiprole, linezolid, vancomycin, and daptomycin against community-associated and hospital-associated methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2008; **52**: 2974–6.

9 Deshpande LM, Jones RN. Bactericidal activity and synergy studies of BAL9141, a novel pyrrolidinone-3-ylidenemethyl cephem, tested against streptococci, enterococci and methicillin-resistant staphylococci. *Clin Microbiol Infect* 2003; **9**: 1120–4.

10 EUCAST. *Clinical Breakpoints*. http://www.srga.org/eucastwt/MICTAB/index.html (3 December 2009, date last accessed).

11 Lodise TP Jr, Pypstra R, Kahn JB *et al*. Probability of target attainment for ceftobiprole as derived from a population pharmacokinetic analysis of 150 subjects. *Antimicrob Agents Chemother* 2007; **51**: 2378–87.

12 Hersh AL, Chambers HF, Maselli JH *et al*. National trends in ambulatory visits and antibiotic prescribing for skin and soft-tissue infections. *Arch Intern Med* 2008; **168**: 1585–91.

13 Gustafson JE, Wilkinson BJ. Lower autolytic activity in a homogeneous methicillin-resistant *Staphylococcus aureus* strain compared to derived heterogeneous-resistant and susceptible strains. *FEMS Microbiol Lett* 1989; **50**: 107–11.

J Antimicrob Chemother 2010
doi:10.1093/jac/dkp458
Advance publication 18 December 2009

Prosthetic hip joint infection with a *Streptococcus agalactiae* isolate not susceptible to penicillin G and ceftriaxone

Christiane Gaudreau[1,2]*, René Lecours[3], Johanne Ismaïl[4], Simon Gagnon[5], Louise Jetté[4] and Michel Roger[2,5]

[1]Microbiologie médicale et infectiologie, Centre hospitalier de l'Université de Montréal (CHUM)-Hôpital Saint-Luc, 1058 rue Saint Denis, Montréal, Québec, Canada, H2X 3J4; [2]Département de microbiologie et immunologie, Université de Montréal, CP 6128 succ. Centre-Ville, Montréal, Québec, Canada, H3C 3J7; [3]Médecine interne, CHUM-Hôpital Saint-Luc, 1058 rue Saint Denis, Montréal, Québec, Canada, H2X 3J4; [4]Laboratoire de santé publique du Québec/Institut national de santé publique du Québec (LSPQ/INSPQ), 20045 chemin Sainte-Marie, Sainte-Anne-de-Bellevue, Québec, Canada, H9X 3R5; [5]Microbiologie médicale et infectiologie, CHUM-Hôpital Notre-Dame, 1560 rue Sherbrooke Est, Montréal, Québec, Canada, H2L 4M1

*Corresponding author. Tel: +1-514-890-8305, ext. 36209; Fax: +1-514-412-7412; E-mail: christiane.gaudreau.chum@ssss.gouv.qc.ca

Keywords: invasive infection, penicillin G non-susceptible, group B *Streptococcus*, PBP

Sir,

Streptococcus agalactiae [group B *Streptococcus* (GBS)] colonize and cause various infections in neonates and adults. GBS are reported to be universally susceptible to penicillin G.[1] Four recent studies documented amino acid substitutions in penicillin-binding protein (PBP) in GBS clinical isolates with increased penicillin MICs.[2–5] Here we report a case of invasive penicillin G-non-susceptible GBS infection.

In 2002, a 55-year-old woman with a history of treated ovarian carcinoma had a right hip prosthesis for a fractured femoral neck. In 2004, the culture of the articular fluid from the right hip was positive for GBS. The patient was treated intravenously with 12×10^6 units of penicillin G Na daily for 6 weeks followed by prolonged oral therapy with 300 mg of penicillin V every 24 h. In 2007, the culture of the pus, from a para-articular collection near the right hip, grew GBS, and penicillin V was increased to 600 mg every 8 h. In 2008, the abscess was drained without surgical debridement, the culture of the pus was negative and the patient was treated with 500 mg of cefadroxil every 12 h for 14 days. Two months later, the technetium and gallium scans were negative for infection, the sedimentation rate and protein electrophoresis were normal and the patient had a normal right hip exam.

Identification of the two GBS isolates was confirmed at the provincial reference laboratory LSPQ/INSPQ. The susceptibility of GBS isolated in 2004 (GBS 2004) and 2007 (GBS 2007) was tested at Hôpital Saint-Luc (Montréal) by Etest (oxacillin, ampicillin and meropenem), by the CLSI disc diffusion method with linezolid 30 μg discs and at LSPQ/INSPQ by the CLSI microdilution method with penicillin G, ceftriaxone, erythromycin, clindamycin, levofloxacin, chloramphenicol and vancomycin.[1,6] MICs of 11 antimicrobial agents for the two GBS isolates are reported in Table 1. The GBS 2007 was not susceptible to ceftriaxone with increased MICs of three dilutions and to penicillin G, ampicillin and oxacillin with increased MICs of two dilutions. MICs of meropenem, even if still susceptible, increased from 0.03 mg/L for GBS 2004 to 0.25 mg/L for GBS 2007. The two GBS isolates were susceptible to erythromycin, clindamycin, vancomycin, levofloxacin, chloramphenicol and linezolid, but were resistant to tetracycline. β-Lactamase was negative for both GBS isolates with nitrocefin discs (BD BBL Cefinase discs). PFGE showed that the two GBS isolates were identical.

Table 1. MICs (mg/L) of antimicrobial agents for GBS isolated in 2004 and 2007

	GBS 2004	GBS 2007	CLSI S	CLSI R
Penicillin G	0.06	0.25	≤0.12	NA
Ceftriaxone	0.12	1	≤0.5	NA
Oxacillin[a]	1	4	NA	NA
Ampicillin	0.12	0.5	≤0.25	NA
Meropenem	0.03	0.25	≤0.5	NA
Erythromycin	0.06	0.06	≤0.25	≥1
Clindamycin	0.06	0.12	≤0.25	≥1
Tetracycline	32	32	≤2	≥8
Vancomycin	0.25	0.5	≤1	NA
Levofloxacin	0.5	0.5	≤2	≥8
Chloramphenicol	4	4	≤4	≥16

GBS, group B *Streptococcus*; CLSI S and CLSI R, MIC breakpoints for susceptibility (S) and resistance (R);[1] NA, not available.
[a]No breakpoints for GBS, but breakpoints for *Staphylococcus* spp. are: ≥4 mg/L, resistant; and ≤2 mg/L, susceptible.