Intraphagocytic Activities of Ceftobiprole vs. Conventional Cephalosporins against Methicillin-Sensitive (MSSA) and Methicillin-Resistant S. aureus (MRSA)

S. Lemaire,[a] F. Van Bambeke,[a] Y. Glupczynski[b] and P.M. Tulkens[a]

[a]Unité de pharmacologie cellulaire et moléculaire, Université catholique de Louvain, Brussels;
[b]Laboratoire de microbiologie, Cliniques universitaires UCL Mont-Godinne, Yvoir; Belgium

Sandrine Lemaire
Pharmacologie cellulaire et moléculaire
UCL 73.70 av. Mounier 73
1200 Brussels – Belgium
tel.: +32-2-764.73.75
fax: +32-2-764.73.73
sandrine.lemaire@uclouvain.be

A-1438

Abstract

Objectives: We have shown recently that meropenem and cloxacillin recover activity against MRSA when these are thriving in macrophages owing to the acid pH prevailing in phagolysosomes (AAC 2007, 51:1627-1632). Our objective was to determine the susceptibility of MSSA and MRSA to ceftobiprole (a new cephalosporin active against MRSA) in broth (at acid and neutral pH) and after phagocytosis by macrophages, in comparison with conventional cephalosporins of different generations.

Methods: MICs were determined in pH-adjusted MHB by micro-dilution. Intracellular activities were determined in human THP-1 macrophages (24 h change in post-phagocytosis inoculum [delta log CFU]) incubated with an extracellular drug concentration corresponding to the reported human Cmax (see details in AAC 2006; 50:841-851).

Results:

Drugs	Cmax (mg/L)	MSSA ATCC 25923			MRSA ATCC 33591		
		MICs (mg/L)		Delta log cfu (24 h)	MICs (mg/L)		Delta log cfu (24 h)
		pH 7.4	pH 5.5		pH 7.4	pH 5.5	
None				+ 1.7 ± 0.1			+ 2.2 ± 0.1
Ceftobiprole	58	0.5	0.25	-1.0 ± 0.1	2	0.5	-0.9 ± 0.1
Cephalexin	18	0.1	0.5	-0.8 ± 0.1	128	8	+1.8 ± 0.1
Cefuroxime	80	0.5	0.125	-0.8 ± 0.1	> 256	1	+0.2 ± 0.1
Ceftazidime	180	8-16	4	+0.6 ± 0.1	128	4-8	+0.8 ± 0.1
Cefepime	130	2	1	-0.8 ± 0.1	256	4	+0.4 ± 0.1

Acid pH only partially restored the activity of conventional cephalosporins towards MRSA, the intraphagocytic growth of which was not efficiently controlled by these drugs. In contrast, ceftobiprole showed similar activities against MSSA and MRSA at acid pH in broth and intracellularly.

Conclusions : Ceftobiprole shows intraphagocytic activity against MRSA due to its large activity at acid pH.

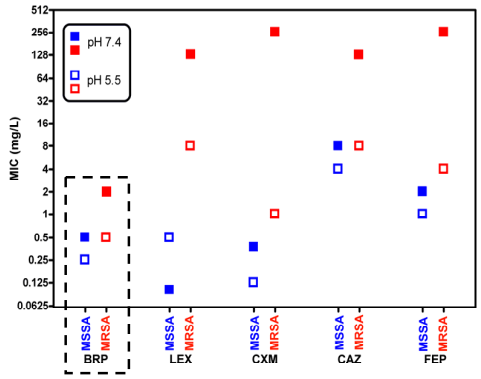
Background

Intracellular survival is often considered as an important determinant in the recurrent and relapsing character of staphylococcal infections. The selection of appropriate agents to eradicate intracellular *S. aureus* remains, however, challenging, since antibiotic activity is usually evaluated against extracellular bacteria only.

We recently showed that β-lactams (meropenem and cloxacillin) recover activity against MRSA when tested towards bacteria phagocytozed by human THP-1 macrophages, and ascribed this unanticipated effect to the acidic pH prevailing in the phagolysosomes containing viable bacteria.[1] This prompted us to evaluate the activity of ceftobiprole (BPR), a new anti-MRSA cephalosporin with tight binding to PBP2a,[3] towards MRSA in acid broth as well as after phagogytosis, in comparison registered cephalosporins of different generations.

Results

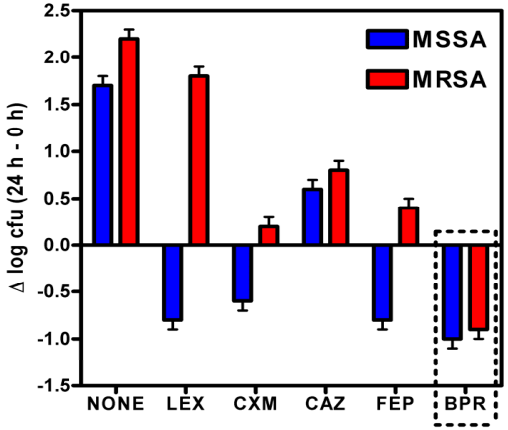
1 Influence of pH on the cephalosporins susceptibilities



MICs were determined in broth against **MSSA ATCC 25923** and **MRSA ATCC 33591**. Antibiotics used under this study are the following : cephalexin (LEX), cefuroxime (CXM), ceftazidime (CAZ), cefepime (FEP) and ceftobiprole (BPR).

For registered cephalosporins, acid pH only partially restored activity towards MRSA (compared to MSSA [for cephalexin (LEX), activity was even lower at acid pH]). In contrast, ceftobiprole (BPR) showed low MICs against both MSSA and MRSA at neutral pH, with further decrease of 1 to 2 dilutions at acid pH.

2 Intraphagocytic activities of antibiotics



Intracellular activity, expressed as a change of cfu per mg of cell protein after 24 h, was determined against **MSSA ATCC 25923** and **MRSA ATCC 33591** phagocytosed by human THP-1 macrophages and using a fixed concentration of drugs (corresponding to their human C_{max}; in mg/L: LEX, 18; CXM, 80; CAZ, 180; FEP, 130; BPR, 58).

Ceftobiprole (BPR) caused a marked decrease of cfus of both intraphagocytic MRSA and MSSA. In contrast, the other cephalosporins tested were unable to prevent the intracellular growth of MRSA while LEX and FEP showed similar activities to BPR for MSSA.

Methods

Susceptibility testing :

MICs were determined by micro-dilution method in Mueller-Hinton broth.

Determination of the intracellular antibiotic activity[1-2]:
Cells were infected with preopsonized bacteria (1 h incub. with normal serum at 37°C), washed with phosphate-buffered saline, and incubated for 45 minutes with gentamicin (50 mg/Liter) to eliminate non-adherent and non-internalized bacteria. Infected cells were then exposed for 24 h to antibiotics at a concentration corresponding to the plasma C_{max} reached in patients treated with conventional dosages (control cells were maintained in the continuous presence of gentamicin [0.5 x MIC] to prevent the extracellular growth of bacteria released from dead cells).

Conclusions

Ceftobiprole, at the concentration tested, proved effective towards intraphagocytic MRSA in this model, probably in relation with its low MIC due to intrinsic activity against PBP2a and a favorable effect of acid pH.

References

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Acknowledgments

S. L. is *Boursière* of the Belgian *Fonds pour la Recherche dans l'Industrie et l'Agriculture* (*FRIA*), and F.V.B. *Maître de Recherches* of the Belgian *Fonds de la Recherche Scientifique* (*FRS-FNRS*). This work was supported by the Belgian *Fonds de la Recherche Scientifique Médicale* (grants no. 3.4.639.04 et 597.06) and by a grant-in-aid from Johnson & Johnson Pharmaceutical Research & Development.

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