Carbapenems are Active against Intracellular MRSA

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ABSTRACT

I. Background

Reccurent *S. aureus* infection may be related to intracellular persistence. In macrophages, *S. aureus* is localized in phagolysosomes where pH is acidic (~5.5). MRSA regain sensitivity to β-lactam when exposed to acidic pH (AAC 2:350-355,1972). We have therefore examined the activity of OXA, MEM and ETP against intracellular MRSA in comparison with MSSA.

II. Methods

MIC were determined by geometric dilutions in MH broth. Intracellular activity was measured in THP-1 macrophages (ICAAC 2004, abstract no. A-1487) at an extracellular concentration corresponding to human Cmax (OXA, 63 mg/L ; MEM, 50 mg/L; ETP, 155 mg/L).

III. Results

	MSSA, ATCC 25923			MRSA, ATCC 33591			
	MIC (mg/L)		Δlog 24h (at Cmax) (A)	MIC (mg/L)		Δlog 24h (at Cmax) (B)	p (A-B)
	7.4	5.5		7.4	5.5		
None			1.2 ± 0.1			1.3 ± 0.1	NS
OXA	0.25	0.06	-1.7 ± 0.1	256	0.25	0.5 ± 0.1	< 0.05
MEM	0.125	0.06	-0.9 ± 0.1	16	0.125	-0.5 ± 0.1	< 0.05
ETP	0.125	0.06	-1.2 ± 0.1	32	0.125	-1 ± 0.2	NS

Acidic pH markedly increased MRSA sensitivity to b-lactams. MEM and ETP (at Cmax), but not OXA, showed significant activity towards intracellular MRSA.

IV. Conclusion

Intraphagocytic MRSA are susceptible to the carbapenems tested, perhaps in relation to their exposure to acid pH and to the concomitant decrease of MIC (conferring to ETP a similar activity against intraphagocytic MRSA and MSSA).

INTRODUCTION

MRSA show a high level of resistance to β-lactams, due to the expression of PBP2a, a modified PBP encoded by the *mecA* gene and presenting a reduced affinity for β-lactams. Over the last years, these strains have widely spread both in hospitals and in the community, causing difficult-to-treat infections.

Pioneer work showed that β-lactams activity against *S. aureus* was restored at acidic pH[1]. This may be of interest for infections by *S.aureus* developing in acidic environments. In particular, *S. aureus* is able to survive and multiply in the phagolysosomal compartments of phagocytic cells (where pH is acidic). We have recently shown that β-lactams do exert intracellular activity against *S. aureus*[2].

AIM OF THE STUDY

- To assess the influence of acidic pH on the activity of OXA, MEM and ETP
- To study the activity of these drugs against intraphagocytic forms of MSSA and MRSA

METHODS

- Minimal Inhibitory Concentrations (MICs) were determined by broth micro-dilutions method, in MHB (NaCl 2%) adjusted to pH 7.4 or 5.5.
- Intracellular activity was assessed in THP-1 human macrophages[2]. Cells were infected with opsonized MSSA (ATCC 25923) or MRSA (ATCC 33591) during 1 h at 37°C, washed with PBS and incubated during 1 h with gentamicin ([MSSA, 50 mg/L ; MRSA, 100 mg/L]) to eliminate non phagocytosed and non-firmly adherent bacteria. Cells were then incubated for 24 h with OXA, MEM and ETP.

RESULTS

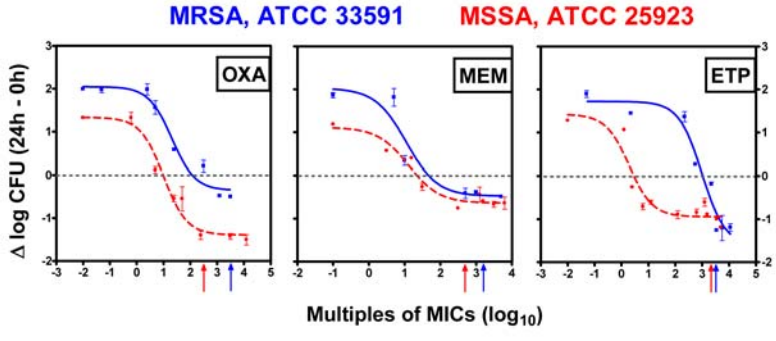
Intrinsic activity

	MSSA ATCC 25923		MRSA ATCC 33591	
	7.4[a]	5.5[a]	7.4[b]	5.5[a]
OXA	0.15	0.02	256	0.2
MEM	0.15	0.1	16	0.1
ETP	0.11	0.04	32	0.04

[a] Arithmetic dilutions (n = 3), [b] Geometric dilutions.

- Against MSSA, MICs of OXA, MEM and ETP were similar and slightly lower at acidic pH.
- Against MRSA, MIC were high at neutral pH. At acidic pH, they became similar to those observed for MSSA

Intracellular activity



Concentration-killing curves of OXA, MEM and ETP towards MSSA and MRSA in a THP-1 model of infection. The abscissa shows the initial concentration of antibiotics in multiples of their MIC (determined in broth adjusted at pH 5.5). The arrow on the x axis point the multiples of MIC corresponding to the Cmax tested (63, 50 and 155 mg/L for OXA, MEM and ETP respectively) for MSSA (■) and MRSA (■). The ordinate shows the change in CFUs (log_{10}) per mg of cell protein observed after 24 h incubation in comparison with the original inocula (horizontal broken lines). All values are mean ± SEM (n=3).

The intracellular activity of β-lactams developed on a concentration-dependent manner when examined over a wide range of concentrations (classical sigmoidal dose-effects relationships).

OXA and ETP were active at lower concentration against intracellular MSSA than against intracellular MRSA (static effects obtained at 10 x MIC vs 125 x MIC for OXA, 4x MIC vs 1000 x MIC for ETP). MEM was almost as active against both strains for concentrations ≥ 10 x MIC (static effect obtained at 25x MIC vs 50X MIC for MSSA and MRSA, respectively).

At concentrations corresponding to their respective Cmax, OXA was static against MRSA while MEM caused a low decrease in intracellular bacterial counts (~ 0.5 log) and ETP was slightly more active (~ 1 log decrease of CFU).

CONCLUSIONS

- Extracellularly, acidic pH improved the activity of β-lactams,
  - modestly against MSSA
  - markedly against MRSA
- Intracellularly, β-lactams show activity against MSSA and, at higher concentrations, against MRSA, suggesting a key role of the phagolysosomal environment in the capacity of β-lactams to act upon MRSA.
- Bactericidal effects were obtained upon exposure to clinically relevant concentrations for Carbapenems only.

REFERENCES

1. Sabath L.D., Wallace S.J. and Gerstein D.A., Suppression of Intrinsic Resistance to Methicillin and Other Penicillins in *Staphylococcus aureus*, Antimicrob.Ag. Chemother., 2(1972), 350-355.

2. Lemaire and al., Activity of three {beta}-lactams (ertapenem, meropenem and ampicillin) against intraphagocytic *Listeria monocytogenes* and *Staphylococcus aureus*. J. Antimicrob. Chemother. 55(2005):897-904