Contrasting effect of acid pH on the extracellular and intracellular activities of gentamicin and oxacillin against *Staphylococcus aureus*

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UCL Université catholique de Louvain

Mailing address: Pierre Baudoux
Unité de pharmacologie cellulaire et moléculaire
UCL 7370, avenue Mounier 73
1200 Brussels – Belgium
e-mail: pierre.baudoux@facm.ucl.ac.be

P. Baudoux, N. Bles, S. Lemaire, M.-P. Mingeot-Leclercq, P. M. Tulkens, F. Van Bambeke*

Unité de Pharmacologie cellulaire et moléculaire, Université Catholique de Louvain, Brussels, Belgium

ABSTRACT

Background and Aim

S. aureus is capable of surviving in mild acidic pH environments, which includes phagolysosomes. Acidity, however, affects also antibiotic activity. We have compared the influence of acid pH on the extracellular and intracellular activities of (a) oxacillin (OXA), a beta-lactam known for its poor accumulation in cells but for an enhanced activity at acid pH; and (b) gentamicin (GEN), which accumulates in phagolysosomes but shows a marked decrease of activity at acid pH.

Methods

MICs were determined in MH Broth adjusted to pH 5.0 to 7.4. Extracellular (MH broth) and intracellular (THP-1 macrophages [Barcia-Macay *et al*., AAC, 50:841, 2006]) activities (expressed as difference in CFU compared to controls) were examined at 24h using a wide range of concentrations to obtain full pharmacological response (Emin, static concentration, Emax).

Results

MICs are shown in Table. Activity was always concentration-dependent (sigmoid dose-response; R2>0.9), with Emax reaching - 5 log (limit of detection) extracellularly and - 1 log intracellularly. Static concentrations are shown in the Table.

		GEN			OXA		
			static conc.			static conc.	
condition	pH	MIC	mg/L	X MIC	MIC	mg/L	X MIC
extracellular	7.4	0.2	0.23	1.15	0.25	0.27	1.07
	5.0	14.5	20.89	1.41	0.03	0.02	0.56
intracellular			0.32	1.58		0.20	1.02

Conclusion

Acid pH has contrasting effects on GEN and OXA activity in broth, but their static concentrations remain close to the corresponding MIC. Intracellularly, OXA and GEN seem equipotent. The negative effect of acid pH for GEN is probably compensated by its larger accumulation, whereas the opposite combination of effects may take place for OXA.

Background and Aim

- *Staphylococcus aureus* is a widespread pathogenic bacterium capable of surviving and multiplying in hostile environments, showing a high tolerance to variations in pH. This confers an advantage for colonizing body sites characterized by a mild acidic pH or to thrive intracellularly in compartments such as phagolysosomes of phagocytic cells.
- Acid environment may also impair the activity of many antibiotics.
- In the present work, we have used gentamicin and oxacillin to compare the influence of pH towards *S. aureus* in broth and to examine their activities against its intracellular forms. We used a model [2] in which bacteria and cells are exposed to a wide range of drug concentrations for up to 24 h, allowing us to draw pharmacological as well as potentially clinically-meaningful conclusions.

Methods

Bacterial strain and determination of extracellular activity of antibiotics

All experiments were performed using a methicillin-susceptible strain of *S. aureus* (ATCC 25923) (ATCC, Manassas, VA). Killing curve experiments were performed by incubating bacteria in pH-adjusted MH broth for 24 h in presence of antibiotic.

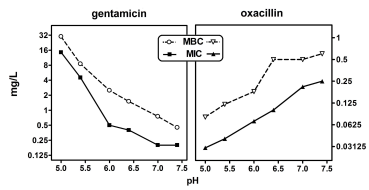
Cell infection and determination of intracellular activity and of cellular accumulation of antibiotics

All experiments were conducted with THP-1 macrophages, infected with opsonized *S. aureus* (ATCC 25923)[1,2]. Cell-associated antibiotics were assayed by microbiological method (disk diffusion).

Data presentation (Figure 2): all data are presented as dose-effect relationships (log Δ CFU vs. log drug concentration) and a sigmoidal function (Hill coefficient = 1) fitted to the data to calculate the E_{50} (relative potency) and the E_{max} (maximal effect) parameters of the response [1,2].

Results

Figure 1 : MIC and MBC of gentamicin (left) and oxacillin (right) as a function of the pH of MH broth (MIC) or agar (MBC). Values are arithmetical means of 4 determinations.



Acid pH { **increases MIC/MBC of gentamicin** / **decreases MIC/MBC of oxacillin**

A. pH exerts a negative effect on gentamicin activity by increasing its relative potency (EC_{50}). In contrast, pH decreases EC_{50} of oxacillin. Maximum effect for both drugs (E_{max}) remain unchanged.

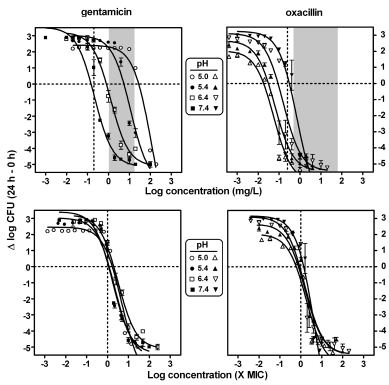
B. Responses become superimposable when plotted as a function of multiples of MIC as determined for each pH value.

C. Intracellularly, E_{max} and, to a lesser extent, EC_{50} are lower than extracellularly.

D. For both gentamicin and oxacillin, drug accumulation in the infected compartment compensate for the effect of pH.

Extracellular activity

Figure 2 : Change in the number of CFU (in log scale) per ml after 24 h incubation of *S. aureus* in broth adjusted to different pH. Data are plotted as a function of the drug concentration expressed in mg/L (first row of panels), and in multiples of the MIC of each drug measured at the considered pH (second row of panels). The zones highlighted in grey correspond to the serum concentrations achieved in patients.



Intracellular activity

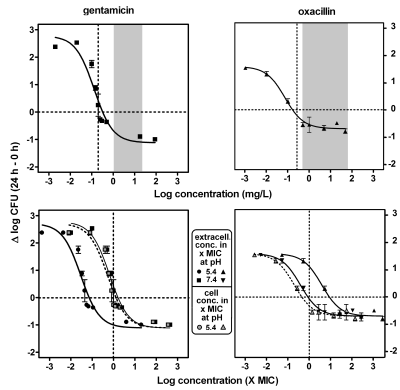


Figure 3 : Change in the number of CFU (in log scale) per mg of cell protein after 24 h incubation of infected THP-1 macrophages with antibiotics. Data are plotted as a function of the drug concentration expressed in mg/L (first row of panels) or in multiples of MIC (second row) of each drug measured at pH 7.4 or at pH 5.4 (open symbols and plain lines), or in lysosomal (gentamicin) or cellular (oxacillin) concentrations expressed in multiples of MIC at pH 5.4 (grey symbols and dotted lines). The zones highlighted in grey correspond to the serum concentrations achieved in patients.

Conclusions

- **We specifically demonstrate that influence of pH is related to a modulation of the relative potencies (EC_{50}) of the antibiotics studied, without adversely affecting the maximal effects (E_{max}) once a suitable concentration is reached. This strongly suggest that the effect of pH is mainly related to modulation of binding and/or accessibility of the drugs to their bacterial target.**
- **These data may help better understanding how the activity of antibiotics could be improved in the clinical arena with respect to intracellular infection and infections in other acidic body sites. Strategies aimed at selecting molecules with low MICs at acidic pH and optimizing exposition of intracellular bacteria taking into account drug accumulation and using extracellular concentrations as high as possible appear as straightforward approaches.**

References

1. Barcia-Macay M., Seral C., Mingeot-Leclercq M. P. *et al. Antimicrob.Agents Chemother.* 2006; **50**, 841-51.
2. Lemaire, S., Van Bambeke, F., Mingeot-Leclercq, M. P. *et al. J.Antimicrob.Chemother.* 2005; **55**, 897-904.

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