asm microbe 2016 JUNE 16–20 • BOSTON asm 2016 ICAAC 2016

SATURDAY-571
Session 188

Limited Maximal Activity without Marked Loss of Potency of Antibiotics against Intracellular Forms of *Staphylococcus aureus* and *Pseudomonas aeruginosa*: An Analysis with Bactericidal Antibiotics from Different Pharmacological Classes in a Pharmacodynamic Model of Human THP-1 Infected Monocytes

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Abstract (edited)

Background
Uptake, survival, and secondary release of bacteria from phagocytes may explain the relapsing and recurrent character of many infections. We have developed a pharmacodynamic model to measure the potency and maximal activity of antibiotics against *S. aureus* and *P. aeruginosa* phagocytized by human THP-1 monocytes. In brief, infected cells are exposed for 24h to drugs concentrations ranging from 0.01 to 100 x the MIC to obtain full concentration-dependent responses and calculate pertinent pharmacodynamic parameters based on Hill equation (sigmoidal dose-response).

Methods
Data obtained with various strains of susceptible *S. aureus* or *P. aeruginosa* strains and 13 antibiotics (all markedly bactericidal in broth) were reviewed. Potency (C_s) was defined as the extracellular concentration causing a static effect (no apparent intracellular bacterial growth; expressed in multiples of the MIC as measured in broth [CLSI method]), and maximal activity (E_{max}) as the decrease of the intracellular CFUs over post-phagocytosis level as extrapolated for an infinitely large extracellular drug concentration.

Results
While all antibiotics showed a C_s (potency) against intracellular bacteria similar or only slightly larger than their MIC in broth (no marked loss of potency), none, except oritavancin [for S. aureus [not illustrated] and meropenem [for P. *aeruginosa*], achieved the CLSI-defined bactericidal effect (≥ 3 log_{10} CFU decrease), denoting a marked loss of maximal efficacy [less negative E_{max}]). Bacteria collected from cells and regrown in broth showed unaltered MICs (no selection of resistant subpopulations).

Conclusion
Across the different classes of bactericidal antibiotic examined, all molecules but two failed to be bactericidal intracellularly although largely maintaining their potency. If also taking place in vivo, this phenomenon may limit the overall efficacy of antibiotic treatments and explain treatment failures.

References

Data were retrieved from the following published works (but only selected data are shown in the poster)

- Buyck *et al.* In vitro Models for the Study of the Intracellular Activity of Antibiotics In "Bacterial Persistence", Molecular Biology Laboratory Protocols Series, J. Michiels and M. Fauvart, editors, 2016, p 147-157. DOI: DOI: 10.1007/978-1-4939-2854-5
- Buyck *et al.* Antimicrob Agents Chemother (2015) 59:4750-4758 - DOI: 10.1128/AAC.00428-15
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- Buyck *et al.* Antimicrob Agents Chemother (2013) 57:2310-2318 -DOI:10.1128/AAC.02609-12
- Melard *et al.* J Antimicrob Chemother (2013) 68: 648–658 - DOI:10.1093/jac/dks442
- Lemaire *et al.* J Antimicrob Chemother (2011) 66:596-607 - DOI:10.1093/jac/dkr159
- Lemaire *et al.* Antimicrob Agents Chemother (2011) 55:649-58 - DOI:10.1128/AAC.01201-10
- Lemaire *et al.* Intern J Antimicrob Agents (2011) 38:52-59 - DOI:10.1016/j.ijantimicag.2011.03.002
- Baudoux *et al.* J of Antimicrob Chemother (2010) 65:1228-1236 - DOI:10.1093/jac/dkq110
- Brinch *et al.* J Antimicrob Chemother (2010) 65:1720-1724 - DOI:10.1093/jac/dkq159
- Lemaire *et al.* Antimicrob Agents Chemother (2010) 54:2549-2559 - DOI:10.1128/AAC.01724-09
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- Barcia-Macay *et al.* Antimicrob Agents Chemother (2006) 50:841-851 - DOI: 10.1128/AAC.50.3.841-851.2006
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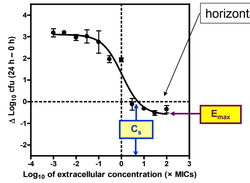
Funding

The present work was performed without support. The original experimental works from which the data were retrieved for analysis were supported by both non-profit and profit-making Institutions and Organizations as indicated in the *ad-hoc* sections of the corresponding papers. These Institutions and Organizations had no role in the collection and interpretation of the data. F.V.B. is Senior Research Associate of the Belgian *Fonds de la Recherche Scientifique* (F.R.S.-FNRS), P.M.T. is emeritus professor and unpaid collaborator.

Background, Model and Data Retrieval

Infections caused *by Staphylococcus aureus* and *Pseudomonas aeruginosa* remain a therapeutic challenge, due, in part, to the ability of these organisms to survive in intracellular compartments of eukaryotic cells. In this context, our laboratory has undertaken to systematically measure and compare the activities of a large array of antibiotics from different pharmacological classes against the intracellular and extracellular forms of both microbes, using a pharmacodynamic model allowing for a quantitative assessment of their concentration-dependent effects in these environments.

In brief, *S. aureus* or *P. aeruginosa* isolates (susceptible to the antibiotics studied) are either (i) placed in broth, or (ii) phagocytized by THP-1 monocytes. Antibiotics are then added to the broth or to the monocytes culture medium for 24 h at concentrations ranging from 0.01 to 100 x the MIC to obtain full concentration-dependent responses and to calculate two pertinent pharmacodynamic parameters based on Hill equation [sigmoidal function] (3). The figure shows a typical example of such as response for intracellular bacteria and the parameters analyzed.



➢ **E_{max} (maximal relative efficacy)**:
CFU change (in log_{10} units) at 24h from the post-phagocytosis inoculum as extrapolated for an infinitely large antibiotic concentration

➢ **C_s (relative potency)**:
extracellular concentration resulting in no apparent bacterial growth from CFU at 0h (CFU at 0h = post-phagocytosis inoculum).

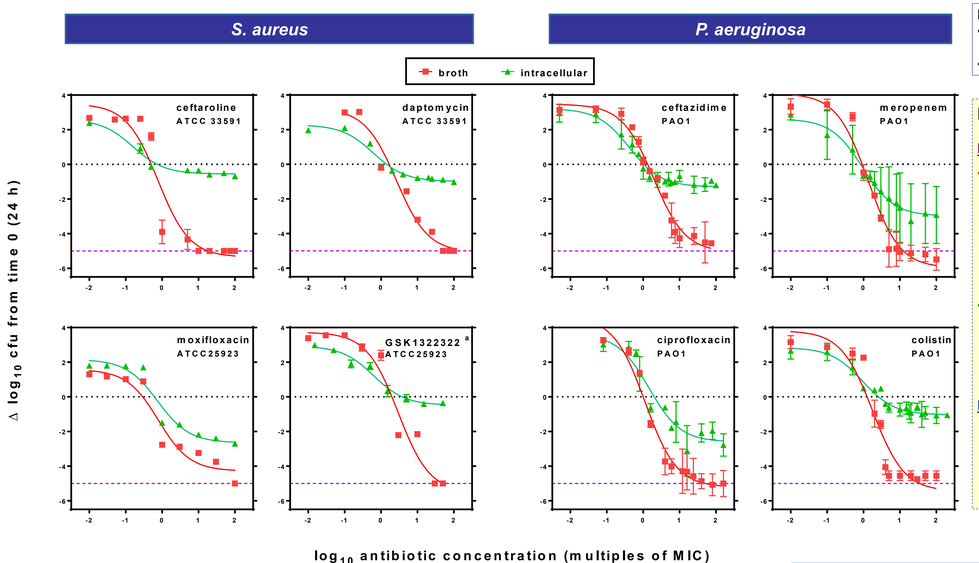
Data obtained with various strains of *S. aureus and P. aeruginosa* (PAO1) for different pharmacological classes (see Results) shown to be known to be bactericidal (> 3 log10 CFU decrease in 24h) in broth were retrieved from our publications and used to calculate, on a homogenous fashion, the corresponding antibiotic relative potencies (C_s) and the maximal relative activities (E_{max}) towards intracellular *vs* extracellular bacteria (see references).

Discussion and Conclusions

- All antibiotics tested shows a sharp decrease of the intracellular maximal relative efficacy (E_{max}) compared to broth, and in most cases, are not bactericidal in cells (less than a 3 log_{10} CFU decrease compared to the post-phagocytosis inoculum) even though all are bactericidal in broth.
- As the calculation of E_{max} assumes an infinitely large extracellular concentrations of antibiotics, the loss of efficacy shown here cannot be due to a simple global lack of diffusion and accumulation of the antibiotics. Conversely, since all antibiotic tested show similar relative potencies (C_s), in the range of their MIC in broth, antibiotics are able to penetrate cells and reach their target (at least in part).
- We also showed (not illustrated here) that bacteria remaining in cells show the same MIC as the original inoculum when collected and grown again in broth and are not Small Colony Variants.

➔ **Intracellular bacteria, for a low but significant proportion of the original inoculum, are probably in a state of non-responsiveness to antibiotics. This may explain why *S. aureus* and *P. aeruginosa* in infections where intracellular inocula are important may prove difficult to treat, as intracellular bacteria remaining viable in cells may reinitiate infection once antibiotic therapy acting on their extracellular forms is discontinued.**

Typical Results



Horizontal dotted lines:
- upper black (·····): initial inoculum (broth inoculation / post-phagocytosis)
- lower purple (– – –): limit of detection

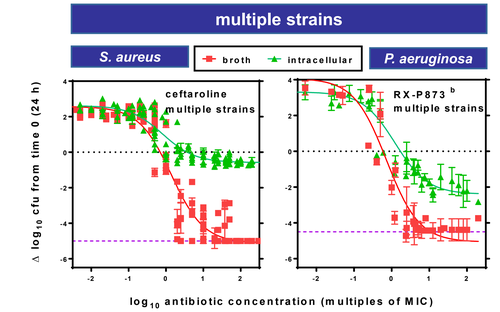
Pharmacodynamic analysis

Maximal relative activity (E_{max})

- **Extracellular bacteria (broth)**
  For all antibiotics shown here, a marked concentration-dependent bactericidal activity was noted with a reduction of the inoculum reaching the limit of detection (4.5 to 5 log_{10} CFU decr.) of concentrations of 10 to 100 x the MIC
- **Intracellular bacteria**
  For all antibiotics, activity is also concentration-dependent but the decrease of CFU is much lower (less negative E_{max}) than for activity in broth

Relative Potency (C_s)

The relative potencies of each antibiotic are roughly similar for extracellular (broth) and intracellular bacteria, and correspond essentially to the MIC in broth (0 of the abscissa log scale)



Differences in E_{max} between broth and intracellular bacteria are observed together with Cs systematically close to MIC in broth (0 on the abscissa log scale) whatever the resistance phenotype of the strains examined:

- ***S. aureus*: strains with ceftaroline MICs ranging from 0.125 to 2 mg/L and with various susceptibility/ resistance phenotypes to other antistaphylococcal antibiotics;**
- ***P. aeruginosa*: strains with RX-P873[b] MICs ranging from 0.5 to 4 and with susceptibility or resistance phenotype to ciprofloxacin**

numerical values of PD parameters for all graphs

antibiotic	strain	E_{max} (log_{10} CFU decr. broth	E_{max} (log_{10} CFU decr. intracellular	C_s (multiple of MIC) broth	C_s (multiple of MIC) intracellular
S. aureus					
ceftaroline	ATCC33591	-5.3	-0.56	0.5	0.8
	multiple strains	-5.1	-0.58	0.7	3.7
daptomycin	ATCC33591	-5.1	-0.99	0.15	1.4
GSK1322322 [a]	ATCC25923	-5.5	-0.48	1.9	3.8
moxifloxacin	ATCC25923	-4.3	-2.7	0.3	0.6
P. aeruginosa					
ceftazidime	PAO1	-5.1	-1.3	1.3	1.0
meropenem	PAO1	-6.0	-3.0	0.9	0.9
colistin	PAO1	-5.4	-1.0	1.2	2.5
ciprofloxacin	PAO1	-5.2	-2.6	1.0	2.0
RX-P853 [b]	multiple strains	-5.1	-2.4	0.6	1.8

[a] a novel peptide deformylase inhibitor with activity against multi-resistant *S. aureus*
[b] a novel inhibitor of bacterial protein synthesis acting a the translation step with broad spectrum activity

This poster will be made available after the meeting at http://www.facm.ucl.ac.be/posters