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Pharmacodynamic Evaluation of 11 Antibiotics against *Pseudomonas aeruginosa* (PAO1) in Broth and in Human THP-1 Monocytes

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Abstract

Objectives: *P. aeruginosa* (Pa) is capable of invading epithelial and phagocytic cells (1), which may play an important role in the initiation and persistence of infections. We have developed a 24 h model of intracellular infection of THP-1 cells by Pa and have used it to examine the activity of the main antipseudomonal antibiotics in comparison with broth.
Methods: Phagocytosis was allowed for 2 h (bacteria/cells ratio:10), extracellular bacteria were eliminated by incubation for 60 min with gentamicin at 100 x MIC, and infected cells (5-7x10^5 CFU/mg cell prot.) incubated with antibiotics (0.01 to 100 x MIC) for 24 h. Activity against bacteria in broth (1x10^6 CFU/ml) was measured in parallel. Activity was expressed as change from the initial inoculum, and data used for fitting conc.-response curves (Hill's equation) to calculate Emax and Cstatic pharmacodynamic parameters (see definition in Table and in ref. 2).
Results: While all antibiotics were cidal in broth (Emax > -3 log CFU), their intracell. Emax was markedly reduced towards intracell. Pa, with CST being the most and fluoroquinolones the least affected. Cstatic remained close to the MIC except for aminoglycosides and CST (10-20-fold increase).
Conclusions: As for *S. aureus* (2), antibiotics are considerably less active and, for aminoglycosides and CST also less potent, against intracellular forms of Pa. compared to bacteria in broth. Intracellular niches may contribute to the difficulty of eradicating Pa *in vivo*, and marked differences between antibiotic classes could be expected in this context.

Background and aim

Pseudomonas aeruginosa, one of the main agents causing pneumonia in cystic fibrosis patients or in ventilated patients in ICUs, is an opportunistic intracellular bacterium. About half of the strains are indeed able to invade and survive within human phagocytes (1). The treatment of such infections is challenging since the activity of antibiotics may differ markedly between the extracellular and intracellular milieus.

In this context, we have set up a model of intracellular infection by *P. aeruginosa,* and used it to determine the intracellular activity of 11 antibiotics currently used in the clinics in comparison with their activity in broth.

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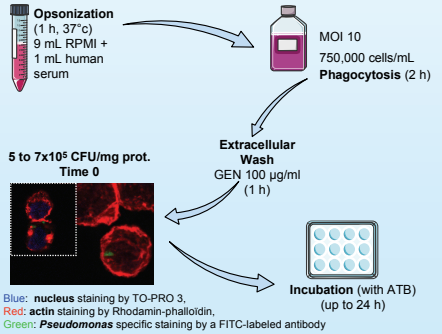
Materials & Methods

Cells and bacterial strain. Experiments were performed with human THP-1 cells, a myelomonocytic cell line displaying macrophage-like activity. *Pseudomonas aeruginosa* wild type strain ATCC PAO1 was used for all experiments.

MIC. All MICs were assayed by the microdilution method. MICs determinations were made in Mueller-Hinton broth (pH 7.4, 24 h).

Cell infection and determination of the intracellular activities of antibiotics. Bacteria were opsonised for 1 h with human serum. Phagocytosis was initiated at a bacteria per macrophage ratio of 10 (2 h at 37°C), followed by elimination of non-phagocytosed bacteria by exposing the cells to 100 mg/L gentamicin (1 h). Cells were then transferred to fresh medium supplemented with increasing concentrations of antibiotics for 24 h. Results, expressed as the change in the intracellular inoculum at 24 h compared to time 0, were used to fit a Hill equation, allowing to calculate pharmacological descriptors of antibiotic activity (static concentration [Cstatic], relative potency [EC50 or 50% effective concentration] and maximal relative efficacy [Emax]; see ref. (2) for a detailed description of these parameters).



References

(1) Kierbel *et al.*, Mol. Biol. Cell 2005,16: 2577-85
(2) Barcia-Macay *et al.*, Antimicrob Agents Chemother., 2006, 50:841-51

Results

Activity of 11 antibiotics against PAO1

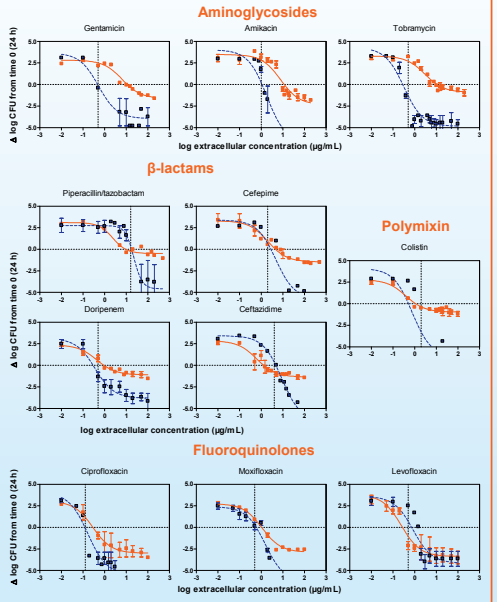


Figure 1. Concentration-killing effects of antibiotics towards *P. aeruginosa.* Blue dotted line: extracellular activity (broth); orange line: intracellular activity (human THP-1 cells). The ordinate shows the change in cfu per mg of cell protein after 24 h compared with the original inoculum. Data are plotted against the weight concentration (µg/mL). All values are mean ± SD (n=3).

Pertinent regression parameters of the dose-response curves illustrated in figure 1

as obtained from sigmoidal function fitted to data sets (Hill equation,R² > 0.90).

Antibiotics	MIC (mg/L)	Extracellular			Intracellular		
		Emax[a] (log cfu)	EC_{50}[b] (mg/L)	Static conc.[c] mg/L (xMIC)	*Emax*[a] (log cfu)	EC_{50}[b] (mg/L)	Static conc.[c] mg/L (xMIC)
Gentamicin	0.5	-4.0 ± 0.4	0.5	0.5 (0.9)	-1.6 ± 0.2	6.5	11.5 (22.9)
Amikacin	1	>5	1.8	1.1 (1.1)	-2.3 ± 0.4	7.9	11.6 (11.6)
Tobramycin	0.5	-4.8 ± 0.2	0.3	0.3 (0.6)	-0.9 ± 0.2	3.0	11.2 (22.4)
Piperacillin/ tazobactam	16	-4.6 ± 0.7	22.3	17.7 (1.1)	-0.5 ± 0.2	2.4	9.2 (2.3)
Cefepime	2	>5	4.8	2.9 (1.4)	-1.5 ± 0.2	2.0	4.6 (4.6)
Ceftazidime	4	>5	9.0	4.8 (0.8)	-1.4 ± 0.2	0.6	1.3 (1.3)
Doripenem	0.5	-3.8 ± 0.4	0.4	0.3 (0.6)	-1.1±0.1	0.9	0.9 (0.9)
Colistin	2	>5	0.6	0.4 (0.2)	-0.9±0.1	0.4	1.1 (0. 6)
Ciprofloxacin	0.125	>5	0.2	0.1 (1.0)	-3.0±0.3	0.3	0.3 (2.3)
Moxifloxacin	0.5	>5	1.5	0.6 (1.2)	-2.9±0.1	1.1	1.0 (2.1)
Levofloxacin	0.5	>5	1.4	1.0 (1.9)	-3.6±0.3	0.2	0.3 (0.5)

[a] relative maximal efficacy: CFU decrease (log10 units) at time 24 h from the corresponding original inoculum, as extrapolated for an infinitely large antibiotic concentration
[b] drug concentration giving a response half-way between E0 and Emax
[c] static concentration (relative potency): concentration resulting no apparent bacterial growth (number of CFU identical to the initial inoculum), as determined by graphical intrapolation.

Conclusions

All antibiotics tested show reduced activity intracellularly, but to different extents :

- Aminoglycosides show a reduction in their maximal efficacy and relative potency (> 10 fold increase in their static concentration).
- β-lactams and colistin also show a reduced maximal efficacy but no marked change in relative potency.
- Fluoroquinolones seem of interest for further investigation, as they reach a bactericidal maximal effect and a potency similar to the extracellular one.

Because they are less susceptible to antibiotics, the intracellular forms of *P. aeruginosa* may contribute to persistence or recurrence of infection, as observed for example in Cystic Fibrosis patients.