Pharmacodynamic evaluation of antibiotics and combinations against *Pseudomonas aeruginosa* (Pa) in broth and in human THP-1 monocytes

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Abstract

Objectives: Pa is capable of invading epithelial and phagocytic cells (1), which may play an important role in the initiation and persistence of infections observed in cystic fibrosis. We have developed a model of intracellular infection of THP-1 cells by Pa and have used it to examine the activity of the main antipseudomonal antibiotics and combination in comparison with broth.

Methods: We used ATCC PAO1 strain. For intracellular activity: phagocytosis was allowed for 2 h (bacteria/cells ratio: 10), extracellular bacteria were eliminated by incubation for 60 min with gentamicin at 100 mg/L, and infected cells incubated over a wide range of conc of antibiotics for 24 h. Activity against bacteria in broth was measured in parallel. Activity was expressed as change from the initial inoculum, and pharmacodynamic parameters [Emax, EC50 and Cstatic (see definition in Table)] were calcutated from the Hill equation of the dose-response. Combinations: Fractional Maximal Effect (FME) method, where AB conc. to be tested are calculated from EC50 and Emax to obtain 0.1, 0.3, 0.5, 0.7, 0.9-fold the Emax. Activity measured for combinations at conc. of AB1 and AB2 giving rise to of 0.1:0.9, 0.3:0.7, 0.5:0.5, 0.7:0.3; 0.9:0.1 effect ratio (2). FME (observed/theoretical effect) for synergy > 1; additivity ~ 1; indifference: < 1; antagonism: < effect of best AB alone.

Results: The table shows that while all antibiotics were cidal in broth (Emax > -3 log CFU), their intracell. Emax was markedly reduced towards intracell. Pa, with fluoroquinolones the least affected. Cstatic remained close to the MIC except for aminoglycosides and CST (10-20-fold increase). Moreover, all combinations tested were synergistic against extracell. Pa (FME > 1) but additive or indifferent against internalized Pa.

Conclusions: Antibiotics are considerably less active against intracellular forms of Pa compared to bacteria in broth. Combining antibiotics may prove useful to act upon intracellul. Pa though to a lesser extent than upon extracell. bacteria. Intracellular niches may contribute to the difficulty of eradicating Pa *in vivo*, and marked differences between antibiotic classes could be expected in this context.

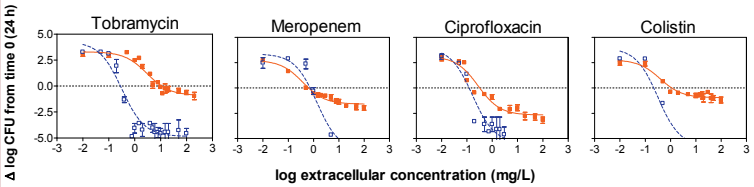
Background and aim

Pseudomonas aeruginosa, one of the main causative agents of pneumonia in cystic fibrosis patients, is an opportunistic intracellular bacterium. About half of the strains is 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 milieu.

In this context, we have we have set up a model of *P. aeruginosa*-infected human monocytes THP-1 cell line and used it to determine the intracellular activity of antibiotics currently used in the clinics in comparison with their activity in broth. We have also examined whether combining antibiotics may improve their activity against intracellular vs. extracellular *P. aeruginosa.*

Results

Activity of antibiotics against extracellular (blue) and intracellular (orange) *P. aeruginosa.*



Antibiotics	MIC (mg/L)	Extracellular *Emax*[a] (log cfu)	Extracellular EC*50*[b] (mg/L)	Extracellular Static conc.[c] mg/L (xMIC)	Intracellular *Emax*[a] (log cfu)	Intracellular EC*50*[b] (mg/L)	Intracellular Static conc.[c] mg/L (xMIC)
Gentamicin	0.5	-4.0 ± 0.4	0.5	0.5 (1)	-1.6 ± 0.2	6.5	11.5 (23)
Tobramycin	0.5	-4.9 ± 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)	-0.6 ± 0.2	3.0	6.3 (0.4)
Cefepime	2	>-5	4.4	2.9 (1.5)	-1.4 ± 0.2	2.0	4.6 (2.3)
Ceftazidime	4	>-5	5.3	4.9 (1.2)	-1.3 ± 0.1	0.6	1.3 (0.3)
Meropenem	1	>-5	1.16	0.8 (0.8)	-1.7±0.2	0.6	0.6 (0.6)
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 (0.8)	-3.0±0.3	0.3	0.3 (2.4)
Levofloxacin	0.5	-4.1 ± 0.4	0.4	0.7 (1.4)	-2.6±0.2	0.3	0.4 (0.8)

[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.

Extracellularly
- ✓ All antibiotics tested were bactericidal (Emax > - 3 log)
- ✓ All static concentrations were close to the MIC

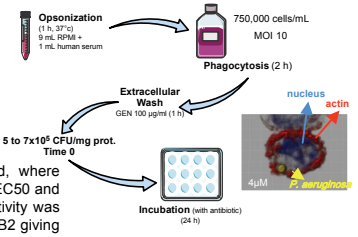
Intracellularly
- ✓ Emax were reduced, with only ciprofloxacin remaining cidal
- ✓ Tobramycin potency was reduced (10-fold increase in Cstatic)

Materials & Methods

Bacterial strain and susceptibility testing. *P. aeruginosa* strain ATCC PAO1 was used. MICs were measured by microdilution in MH broth supplemented with 10% of fetal calf serum.

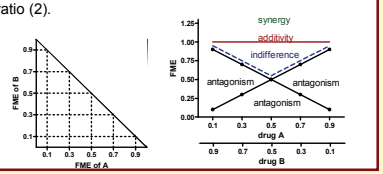
Pharmacodynamics of antibiotics alone.
Extracellular activity was measured in MHB; intracellular activity were measured as described previously in a model of PAO1-infected THP-1 cells (3). PD parameters (Emax [max CFU decrease extrapolated for infinitely large concentration]; EC50 [concentration for which E = ½ Emax]) were calcutated from the Hill equation of the dose-response.

Pharmacodynamics of combinations.
We used the Fractional Maximal Effect (FME) method, where antibiotic concentrations to be tested are calculated from EC50 and Emax to obtain 0.1, 0.3, 0.5, 0.7, 0.9-fold the Emax[a]. Activity was measured for combinations at concentration of AB1 and AB2 giving rise to of 0.1:0.9, 0.3:0.7, 0.5:0.5, 0.7:0.3; 0.9:0.1 effect ratio (2).



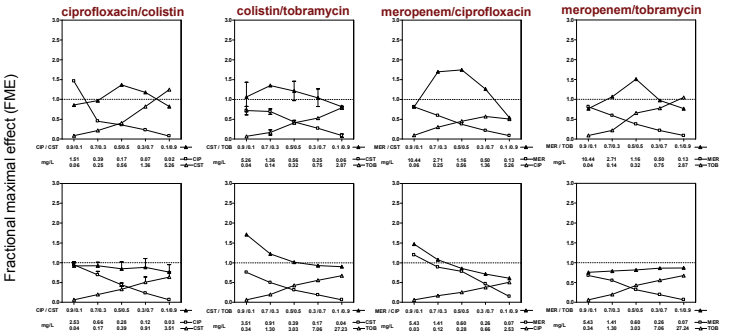
Fractional Maximal Effect (observed/theoretical effect):
synergy > 1; additivity ~ 1; indifference: < 1;
antagonism: < effect of best AB alone

$$C_A = \frac{FME_A \cdot EC_{50A}}{1 - FME_A} \qquad C_B = \frac{FME_B \cdot EC_{50B}}{1 - FME_B}$$



Results

Activity of combinations against extracellular (upper panel) and intracellular (lower panel) *P. aeruginosa.*



Extracellularly
- ✓All combinations were synergistic at least when combined at concentrations generating 0.5 FME for individual drugs

Intracellularly
- ✓ Combinations globally rather show additive effets

Conclusions

All antibiotics tested show reduced activity intracellularly, but to different extents :
- ✓ Tobramycin shows a reduction in its maximal efficacy and relative potency.
- ✓ Meropenem, ciprofloxacin and colistin also show a reduced maximal efficacy but no marked change in relative potency.
- ✓ Only ciprofloxacin reaches a bactericidal maximal effect.

Combining these antibiotics proves highly effective towards extracellular *P. aeruginosa*, and to al lesser extent against intracellular bacteria. Yet, this strategy may help to prevent emergence of resistance.

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

References

(1) Kierbel *et al.*, Mol. Biol. Cell 2005,16: 2577-85
(2) Nguyen *et al.*, AAC 2009, 53: 1443–9
(3) Barcia-Macay *et al.*, AAC 2006, 50:841-51

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