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Activity of antibiotic combinations towards intracellular *Pseudomonas aeruginosa* (Pa) in a model of THP-1 macrophages

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Abstract

Objectives: Pa has been shown to be poorly susceptible to most antibiotics (AB) except fluoroquinolones (> -2 log CFU in 24 h) in a model of infected THP-1 cells (ICAAC 2010 A1-1395). We have now examined whether combining antibiotics may improve their activity against intracell. vs. extracell. Pa.

Methods: strain: ATCC PAO1. AB alone: extracell. and intracell. activity measured over a wide range of conc.; PD parameters (Emax [max CFU decrease extrapolated for infinitely large conc.]; EC50 [conc. for which E = ½ Emax]) 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 (AAC 2009, 53: 1443–9). Fractional Maximal Effect (observed/theoretical effect) for synergy > 1; additivity ~ 1; indifference: < 1; antagonism: < effect of best AB alone.

Results: The table shows the PD parameters for AB alone and the FME for a 0.5:0.5 ratio. All combinations tested were synergistic against extracell. PAO1 (FME > 1) but additive or indifferent against internalized PAO1 (similar conclusion of other conc. ratios in the combination).

Antibiotics	Extracellular		Intracellular		Combinations[c] (AB1/AB2)	FME[d]	
	E_{max}[a]	EC_{50}[b]	E_{max}[a]	EC_{50}[b]		Extracellular	Intracellular
Tobramycin (TOB)	-4.85	0.32	-0.90	3.03	MER/TOB	1.61	0.78
Meropenem (MER)	> -5	1.16	-1.65	0.60	MER/CIP	1.32	0.91
Ciprofloxacin (CIP)	> -5	0.17	-2.67	0.27	CST/CIP	1.50	0.59
Colistin (CST)	> -5	0.58	-0.97	0.39	CST/TOB	1.66	0.90

[a] maximum decrease in log CFU compared to initial inoculum for an infinitely high concentration in antibiotic
[b] Concentration (in mg/L) causing a reduction of the inoculum half-way between the initial and the maximal (Emax) values, as determined by graphical intrapolation
[c] antibiotics combined at concentrations giving rise to 50 % of Emax
[d] Fractional Maximal Effect (observed/theoretical effect) for synergy > 1; additivity ~ 1; indifference: < 1; antagonism: < effect of best AB alone alone.

Conclusions: Combining antibiotics may prove useful to act upon intracellul. Pa, though to a lesser extent than upon extracell. bacteria. Yet this interest should be reinforced when dealing with resistant bacteria, which needs to be further explored in the future.

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Results

Table 1: Pharmacodynamic parameters evaluated from Hill equation of antibiotics alone towards extracellular and intracellular *P. aeruginosa* PAO1 strain.

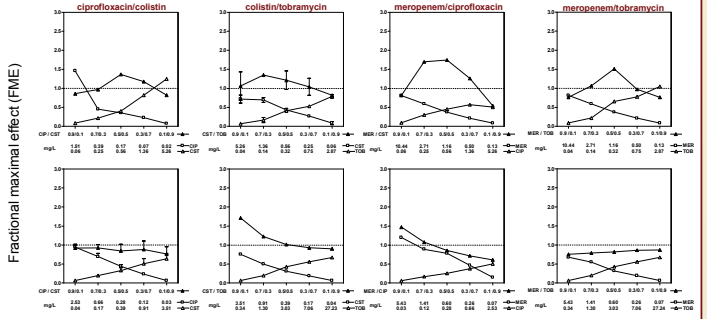
Antibiotics	MIC (mg/L)	Extracellular			Intracellular		
		Emax[a] (log cfu)	*EC50*[b] (mg/L)	Static conc.[c] mg/L (xMIC)	*Emax*[a] (log cfu)	*EC50*[b] (mg/L)	Static conc.[c] mg/L (xMIC)
Tobramycin	0.5	-4.8 ± 0.2	0.3	0.3 (0.6)	-0.9 ± 0.2	3.0	11.2 (22.4)
Meropenem	0.5	> -5	1.16	0.8 (0.8)	-1.6 ± 0.2	0.6	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 (1.0)	-3.0 ± 0.3	0.3	0.3 (2.3)

[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)

Figure 1: 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 show 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

Background and aim

Pseudomonas aeruginosa, one of the main causative agents of pneumonia in cystic fibrosis patients or ventilated 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 shown that *P. aeruginosa* is poorly susceptible to most antibiotics except fluoroquinolones (> -2 log CFU in 24 h) in a model of infected THP-1 cells (ICAAC 2010 A1-1395). We have now examined whether combining antibiotics may improve their activity against intracellular vs. extracellular *P. aeruginosa*.

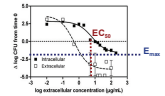
Materials & Methods

Bacterial strain and susceptibility testing. *P. aeruginosa* strain ATCC PAO1 was used. MICs were measured by microdilution in MH broth or in RPMI medium (used for eucaryotic cells culture) 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. 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.

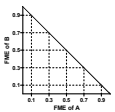
Pharmacodynamics of combinations.

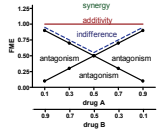
We used the Fractional Maximal Effect (FME) method, where antibiotic concentrations to be tested are calculated from EC50 and Emax to obtain 0.1, 0.3, 0.5, 0.7, 0.9-fold the Emax. Activity was measured for combinations at concentration of AB1 and AB2 giving rise to of 0.1:0.9, 0.3:0.7, 0.5:0.5, 0.7:0.3; 0.9:0.1 effect ratio (3).



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

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





References

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