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Activity of antibiotic combinations towards intracellular *Pseudomonas aeruginosa* (Pa) with different resistance phenotypes 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 24h) in a model of infected THP-1 cells (Buyck et al; AAC 2013). We have now examined whether combining ABs may improve their activity against intracellular vs. extracellular Pa with different resistance phenotypes.
Methods: strains: ATCC PAO1 as reference; clinical strains PA50 (CIP^R), PA291 (MER^R). Activity of ABs alone: extracellular and intracellular activity measured over a wide range of concentrations; PD parameters (E_{max} [max CFU decrease extrapolated for infinitely large concentration]; EC_{50} [concentration for which E = ½ E_{max}]) calculated from the Hill equation of the dose-response. Combinations: Fractional Maximal Effect (FME) method, where AB concentrations to be tested are calculated from EC_{50} and E_{max} to obtain 0.1, 0.3, 0.5, 0.7, 0.9-fold the E_{max}. Activity measured for combinations at concentrations 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).
Results: The table shows the PD parameters for AB alone and the FME for a 0.5:0.5 ratio. AB alone: Against extracellular *Pa*, E_{max} were reduced against PA291 strain for all ABs except CIP. EC_{50} were globally close or at low multiples of the MIC. Against intracellular *Pa*, Emax were systematically lower than extracellularly, the reduction being less marked for PA291. EC_{50} were slightly higher than extracellularly for TOB and for MEM against PA291 and slightly lower for CIP against PA50. Combinations: Extracellularly, all combinations are synergistic against PAO1 and PA50; against the MEM^R PA291, combinations were additive when including MEM and synergistic in other cases. Intracellularly, all combinations are additive, or slightly synergistic against PAO1.
Conclusions: Combining antibiotics may prove useful to act upon intracellular *Pa*, though to a lesser extent than upon extracellular bacteria. This interest seems to be maintained against resistant strains.

References

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(2) Buyck *et al.* Pharmacodynamic evaluation of the intracellular activity of antibiotics towards Pseudomonas aeruginosa PAO1 in a model of THP-1 human monocytes. Antimicrob. Ag. Chemother. (2013) 57: 2310-18.
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(4) Nguyen *et al.* Intracellular activity of antibiotics in a model of human THP-1 macrophages infected by a Staphylococcus aureus Small Colony Variant isolated from a cystic fibrosis patient : 2. Study of antibiotic combinations. Antimicrob. Ag. Chemother (2009) 53:1443-49.

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Background and Aims

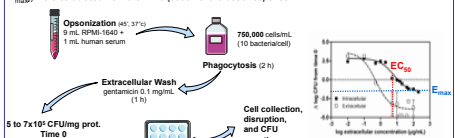
Pseudomonas aeruginosa, one of the main causative agents of pneumonia in ventilated patients or cystic fibrosis patients, is an opportunistic intracellular bacterium. About half of the strains is indeed able to invade and survive within human phagocytes (1). The treatment of such infections is challenging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

In this context, we have 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 (2). We have now examined whether combining antibiotics may improve their activity against intracellular vs. extracellular *P. aeruginosa* strains with different resistance phenotypes.

Methods

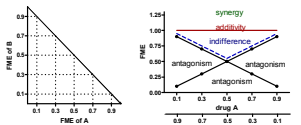
Bacterial strains and susceptibility testing.
• *P. aeruginosa* strain ATCC PAO1 was used as reference. PA50 and PA291 are clinical strains isolated from patients suffering from hospitally-acquired pneumonia (3) and resistant to CIP and MER, respectively.
• MICs were measured in CA-MHB according to the recommendations of CLSI.

Pharmacodynamics of antibiotics alone.
• Dose-kill curve studies were performed in CA-MHB using an initial inoculum: ~10^6 CFU/mL. Samples were collected after 24 h incubations with antibiotics and used for CFU counting.
• Intracellular activity were measured in a model of *Pa*-infected THP-1 cells (1). PD parameters (E_{max} [max CFU decrease extrapolated for infinitely large concentration]; EC_{50} [concentration for which E = ½ E_{max}]) were calculated from the Hill equation of the dose-response.



Pharmacodynamics of combinations.
• We used the Fractional Maximal Effect (FME) method (4), where antibiotic concentrations to be tested are calculated from EC_{50} and E_{max} to obtain 0.1, 0.3, 0.5, 0.7, 0.9-fold the E_{max}. 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.

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



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

Results

Table 1: Susceptibility testing. MICs (mg/L) of TOB, MER, CIP and CST for PAO1 (reference strain), PA50 (CIP^R clinical isolate) and PA291 (MER^R clinical isolate).

Strains [a]	MIC (mg/L)			
	TOB	MER	CIP	CST
ATCC PAO1	1	1	0.125	1
PA50	1	2	16	1
PA291	1	64	0.5	2

[a] ATCC PAO1: wild type reference strain; PA50: clinical isolate resistant to ciprofloxacine, PA291: clinical isolate resistant to meropenem
TOB: tobramycin; MER: meropenem; CIP: ciprofloxacine; CST: colistin.

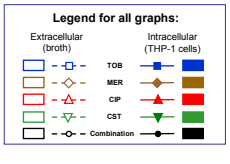


Figure 1: Concentration-response curves of TOB, MER, CIP and CST towards reference strain PAO1 and clinical strains PA50 and PA291. Upper panels: extracellular activity (broth); lower panels: intracellular activity (human THP-1 cells). The ordinate shows the change in cfu per mg of cell protein after 24 h compared with the original inoculum. Data are plotted against the weight concentration (mg/L). All values are mean ± SD (n=3-6).

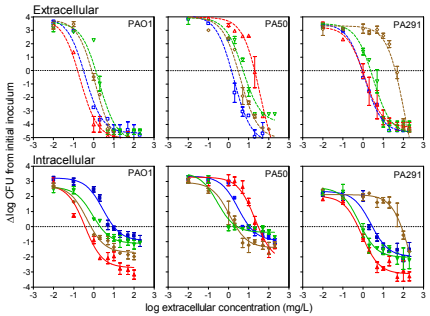
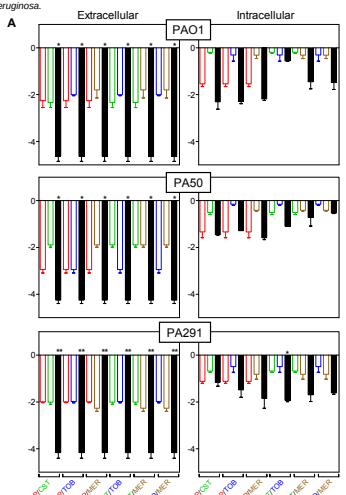
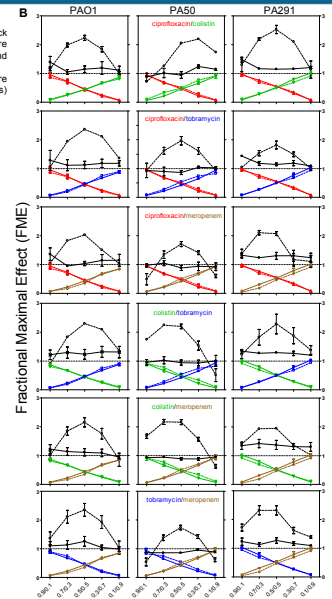


Figure 2: Activity of combinations against *P. aeruginosa* strains.
A. Activity of antibiotic alone (open bars from different colors) and in combination (black bars) at fixed concentrations (giving rise to 50% of their respective E_{max}). Data are expressed in Δlog CFU from initial inoculum, against extracellular (left panel) and intracellular (right panel) *P. aeruginosa*.
B. Activity of antibiotic alone (colored lines) and in combination (black lines). Data are expressed in FME index against extracellular (dotted lines) and intracellular (solid lines) *P. aeruginosa*.





Conclusions

Antibiotics alone:
All antibiotics tested show reduced activity intracellularly for the three strains, but to different extents :
- TOB shows a reduction in its maximal efficacy and relative potency.
- MEM, ciprofloxacin and colistin also show a reduced maximal efficacy but no marked change in relative potency.
- Only CIP reaches a bactericidal maximal effect towards susceptible strains.

Combinations:
- Extracellularly, all combinations are synergistic.
- Intracellularly, synergy is less important and is generally not observed for combinations containing CIP, possibly due to its high intracellular efficacy when used alone.

Antibiotic combination may constitute an appropriate strategy to improve eradication of both extracellular and intracellular *P. aeruginosa*, including resistant strains.

Table 2: Pharmacodynamic parameters (Figs 1 and 2)
For antibiotic alone: calculated from the Hill equation of concentration- kill curves against extracellular and intracellular *P. aeruginosa*.
For combinations: FME indexes calculated for combinations of antibiotics as concentrations allowing to reach 50 % of their Emax

Strains	Model	Antibiotic alone								Combination [c] FME [d] index (Δlog CFU)						
		Emax [a]				EC50 [b]										
		TOB	MER	CIP	CST	TOB	MER	CIP	CST	CIP/CST	CIP/TOB	CIP/MER	CST/TOB	CST/MER	TOB/MER
PAO1	Extra	>-5	>-5	>-5	>-5	0.4	1.2	0.2	1.8	2.2 (-4.6)	2.4 (-4.6)	2.0 (-4.4)	2.3 (-4.6)	2.1 (-4.6)	2.2 (-4.6)
	Intra	-0.9	-1.1	-2.7	-1.7	2.8	0.5	0.4	0.9	1.2 (-2.3)	1.1 (-2.2)	1.1 (-2.2)	1.3 (-0.7)	1.4 (-1.7)	1.3 (-1.7)
PA50	Extra	>-5	>-5	>-5	>-5	2.1	4.3	37.9	5.0	2.1 (-4.4)	2.0 (-4.4)	1.7 (-4.4)	2.2 (-4.4)	2.1 (-4.4)	1.7 (-4.4)
	Intra	-0.9	-0.4	-2.3	-1.1	3.0	2.4	21.0	5.3	0.9 (-1.4)	0.9 (-1.3)	0.9 (-1.5)	0.9 (-1.1)	0.9 (-0.6)	0.9 (-0.5)
PA291	Extra	-3.8	-4.3	>-5	-3.7	0.6	119.9	0.5	2.9	2.4 (-4.2)	1.8 (-4.2)	2.0 (-4.2)	2.2 (-4.2)	1.9 (-4.2)	2.2 (-4.2)
	Intra	-2.0	-2.0	-3.0	-3.1	2.4	222.1	0.6	0.6	1.2 (-1.3)	1.2 (-1.6)	1.2 (-1.9)	1.3 (-1.9)	1.3 (-1.8)	1.3 (-1.6)

[a] maximum decrease in log CFU compared to initial inoculum for an infinitely high concentration in antibiotic as calculated from the Hill equation of the concentration-response curve
[b] Concentration (in mg/L) causing a reduction of the inoculum half-way between the initial and the maximal (Emax) values, as calculated from the Hill equation of the concentration-response curve
[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