Pharmacological effects of tobramycin/macrolides combination on extracellular and intracellular infection by *Pseudomonas aeruginosa*

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Background and objectives

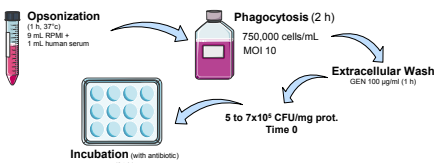
P. aeruginosa (PA) is capable of invading epithelial and phagocytic cells (1), which may play an important role in the initiation and persistence of infections. PA is reported as intrinsically resistant to several antibiotics, including macrolides (ML). Yet ML are currently used with success in clinics to treat cystic fibrosis patients with chronic PA infection. We have shown that ML regain activity against PA in media relevant of the in vivo environment (serum, bronchoalveolar lavage fluid [BAL]) or in culture medium for eukaryotic cells (RPMI-1640) (2).

This study examines the activity of combinations of tobramycin (TOB) and macrolides (azithromycin [AZM] or clarithromycin [CLR]) against intracellular PA (24h infection in THP-1 phagocytic cells) in comparison with extracellular PA grown in broth (MHB) or in RPMI-1640 (RPMI).

Materials and Methods

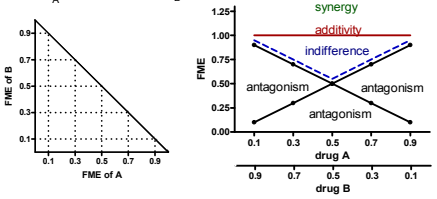
Bacterial strain and susceptibility testing. *P. aeruginosa* strain ATCC PAO1 was used. MICs were measured by microdilution in MH broth or in eucaryotic cell media RPMI-1640 supplemented with 10% of foetal calf serum.

Pharmacodynamics of antibiotics alone. Extracellular activity was measured in MHB or in RPMI-1640 (+ 10 % foetal calf serum); intracellular activity was measured in a model of PAO1-infected THP-1 cells. 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, 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 Emax. Activity was measured for combinations at concentration of antibiotic A and antibiotic B 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).

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



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

Results

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

1. MICs of antibiotics

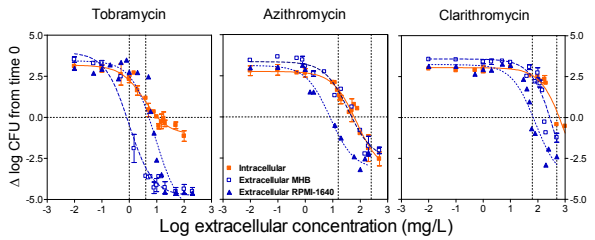
Antibiotics	MIC (mg/L)	
	MHB	RPMI-1640
Tobramycin	1	4
Azithromycin	256	16
Clarithromycin	>512	64

✓MICs of ML were much lower in RPMI than in MHB
✓MIC of TOB was 2 dilutions higher in RPMI

Extracellularly
✓ ML were poorly potent (EC50 very high) but were 6-fold lower in RPMI than in MHB
✓ TOB relative potency was higher in MHB than in RPMI

Intracellularly
✓ Emax and potency were reduced for TOB

2. Dose effect curves



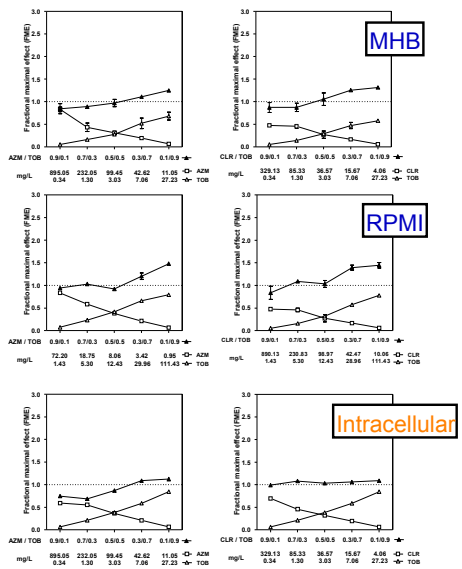
3. Pharmacological parameters

Antibiotics	Extracellular (MHB)		Extracellular (RPMI)		Intracellular	
	$Emax^a$ (Δlog cfu)	$EC50^b$ (mg/L)	$Emax^a$ (Δlog cfu)	$EC50^b$ (mg/L)	$Emax^a$ (Δlog cfu)	$EC50^b$ (mg/L)
Tobramycin	> -4.5	0.4	> -4.5	8.9	-1.1	3.6
Azithromycin	> -4.5	38.9	-3.0	6.1	-3.3	72.3
Clarithromycin	-2.7	510.5	-3.9	82.0	-4.0	851.7

[a] relative maximal efficacy: CFU decrease (log_{10} 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

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

2. Dose effect curves



1. MICs of combinations

Combinations	MIC (mg/L)	
	MHB	RPMI
TOB/AZM[a]	0.5	0.5
TOB/CLR[a]	0.5	0.5

[a] for a combination TOB/macrolide of 10:2 (w/w)

3. Pharmacological parameters

Combinations	FME		
	MHB	RPMI	Intracellular
TOB/AZM[a]	1.31	1.44	1.09
TOB/CLR[a]	1.24	1.47	1.12

[a] for a combination TOB/macrolide of 0.9: 0.1

Extracellularly
✓Combinations showed lower MICs than those of AB alone
✓All combinations were slightly synergistic (FME>1)

Intracellularly
✓ Combinations globally rather show additive effets

Conclusions

The relative potency of antibiotics towards PA is reduced intracellularly as compared to that observed in broth.

For ML, this suggests that the intracellular medium, in contrast to other eukaryotic environments (like BAL, serum, or RPMI-1640), does not allow them to recover their activity against PA. Yet, combining them with TOB, a major antipseudomonal agent, proves synergistic or additive against extracellular and intracellular bacteria.

This warrants further investigations to extend this observation to clinical isolates, including particular phenotypes frequent in cystic fibrosis patients (small colony variants, mucoïdal).

References

(1) Kierbel *et al.*, Mol. Biol. Cell 2005,16: 2577-85
(2) Buyck *et al.*, Clin. Infect. Dis. 2012, 55:534-542
(3) Nguyen *et al.*, AAC 2009, 53: 1443–9

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