Setting-up a model of intracellular infection by *Pseudomonas aeruginosa* for the pharmacodynamic evaluation of antibiotic activity.

J. Buyck[1], O. Jolois[2], P.M. Tulkens[1] and F. Van Bambeke[1]

[1] Pharmacologie cellulaire et moléculaire, Louvain Drug Research Institute, Université catholique de Louvain, Brussels, Belgium
[2] Laboratoire d'histologie humaine, Université de Liège, Liège, Belgium

Mailing address:
F. Van Bambeke
UCL 73.70 av. Mounier 73,
1200 Brussels – Belgium,
francoise.vanbambeke@uclouvain.be

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

BACKGROUND & AIMS

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

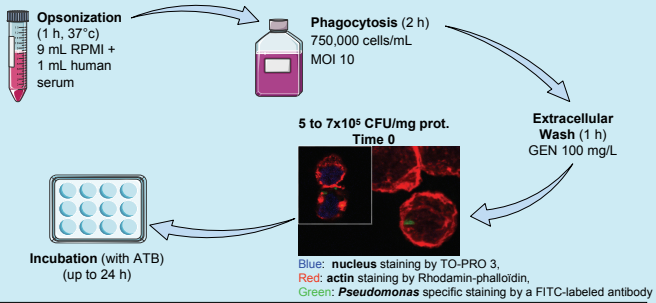
Appropriate models are needed to test for the activity of antibiotics against these intracellular forms. In this context, we have set up a model of intracellular infection by *P. aeruginosa* and used it to determine the intracellular activity of 11 antibiotics currently used in the clinics in comparison with their activity in broth.

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); non-phagocytosed bacteria were then eliminated 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's equation to allow determination of the values of three key 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).



RESULTS

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

as obtained from sigmoidal function fitted to data sets (Hill's equation, $R^2 > 0.90$).

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 (1.0)	-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)	-0.6 ± 0.1	3.3	6.3 (0.4)
Cefepime	2	>5	4.4	2.9 (1.4)	-1.5 ± 0.2	2.0	4.6 (2.3)
Ceftazidime	4	-4.9 ± 0.5	5.3	3.9 (1.0)	-1.3 ± 0.2	0.6	1.3 (0.3)
Doripenem	0.5	-3.8 ± 0.3	0.4	0.3 (0.6)	-1.1 ± 0.1	0.4	0.9 (1.8)
Colistin	2	>5	0.6	0.4 (0.2)	-1.0 ± 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	-4.1 ± 0.5	0.8	0.7 (1.4)	-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.

RESULTS

Activity of 11 antibiotics against PAO1

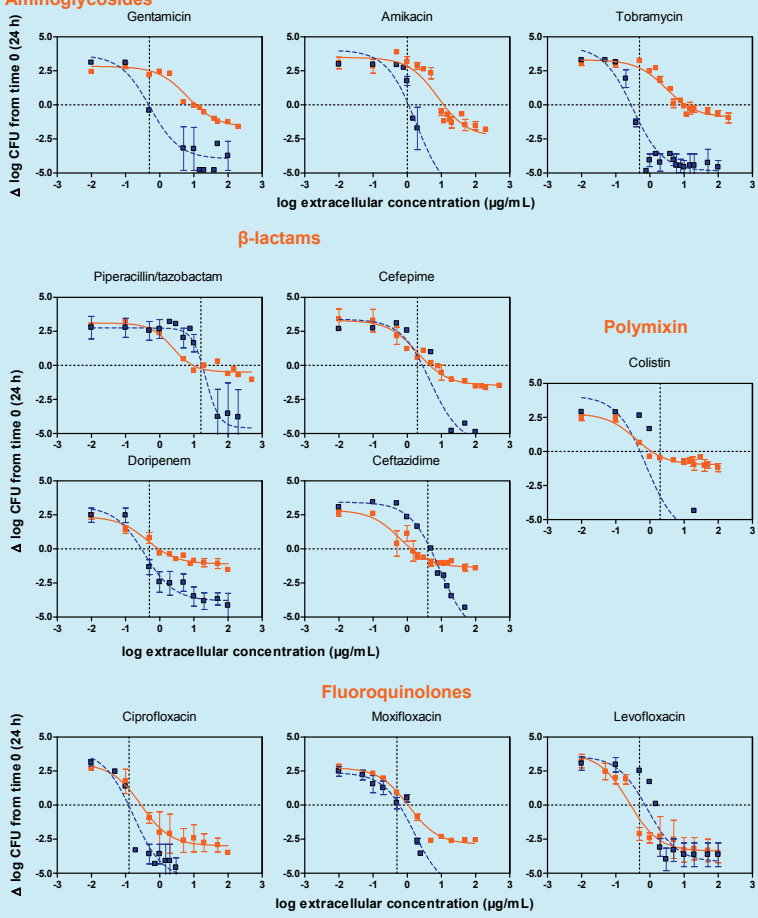


Figure 1. Concentration-killing effects of antibiotics towards *P. aeruginosa.* Blue dotted line: extracellular activity (broth); orange line: intracellular activity (human THP-1 cells). The ordinate shows the change in cfu per mg of cell protein after 24 h compared with the original inoculum. Data are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

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.

This lower activity may contribute to persistence or recurrence of infection as observed for example in Cystic Fibrosis patients.

POTENTIAL APPLICATIONS & KEY BENEFITS

Our data suggest that intracellular niches may contribute to the difficulty of eradicating Pa *in vivo.* Therefore, our model may help in defining which antibiotics may be useful in this context.

REFERENCES

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

ACKNOWLEDGEMENTS:
We thank M.C. Cambier and C. Misson for technical assistance.
This work was supported by the BioWin program of the Region Wallonne.