Setting-up an *in-vitro* model of mature biofilm of *Pseudomonas aeruginosa* to evaluate the activity of antibiotics used to treat chronic lung infection in Cystic Fibrosis patients.

Yvan Diaz Iglesias[1], Muhammad-Hariri Mustafa[1], Paul M. Tulkens[1], Françoise Van Bambeke[1]

[1]Pharmacologie cellulaire et moléculaire, Louvain Drug Research Institute *Université catholique de Louvain*, Brussels, Belgium

Mailing address :
Y. Diaz Iglesias
Av. Mounier 73 (B1.73.05)
1200 Brussels, Belgium
yvan.diaziglesias@uclouvain.be

Introduction

Pseudomonas aeruginosa is a major cause of chronic pulmonary infections in Cystic Fibrosis patients (prevalence near 75%) due to its capacity to grow as biofilms, which are refractive to the action of antibiotics. Appropriate models to evaluate antibiotic activity against biofilms are therefore warranted. Existing models in microplates take a long time (> 10 days) to reach maturity (1).

Aim

Our aim was to set up a model that reaches maturity in a shorter time, in order to evaluate the activity of relevant antibiotics used to treat chronic pseudomonal infections in CF patients.

Methods

PAO1 was used as reference strain. Biofilms were grown in cation-adjusted Mueller-Hinton broth (CA-MHB) using 96 well cell culture microplates (clear, TC surface treatment) for cell culture, with daily renewal of the culture medium over 6 days.

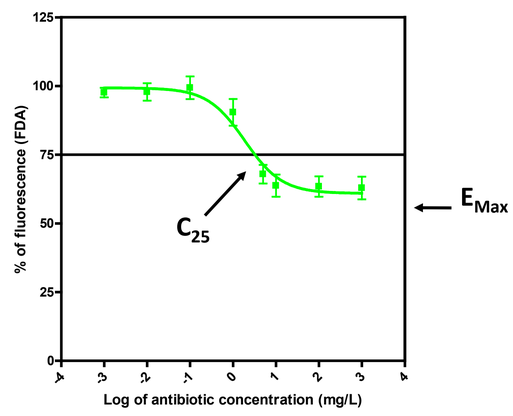
Plates were harvested every day and used to evaluate biofilm biomass by crystal violet staining and total bacterial viability within biofilm by the fluorescein diacetate assay (2).

The same parameters were then evaluated in 4-days old biofilms exposed during 24h to antipseudomonal antibiotics at concentrations ranging from 0.001 to 1000 mg/L in order to obtain full concentration-response curves.

The 5 tested antibiotics were : tobramycin [aminoglycoside], ciprofloxacin [fluoroquinolone], meropenem, ceftazidime [β-lactams], and colistin [polymyxin].

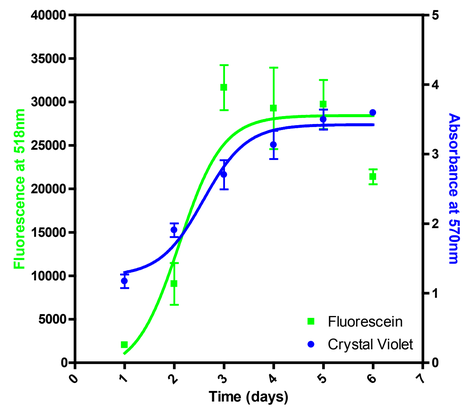
Pharmacodynamic parameters were calculated based on the equation of the sigmoidal regression fitted of the data.

- Maximal efficacy (E_{max}) : reduction in viability/biomass for an infinitely large concentration
- Relative potency (C_{25}) : Concentration reducing of 25% viability/biomass



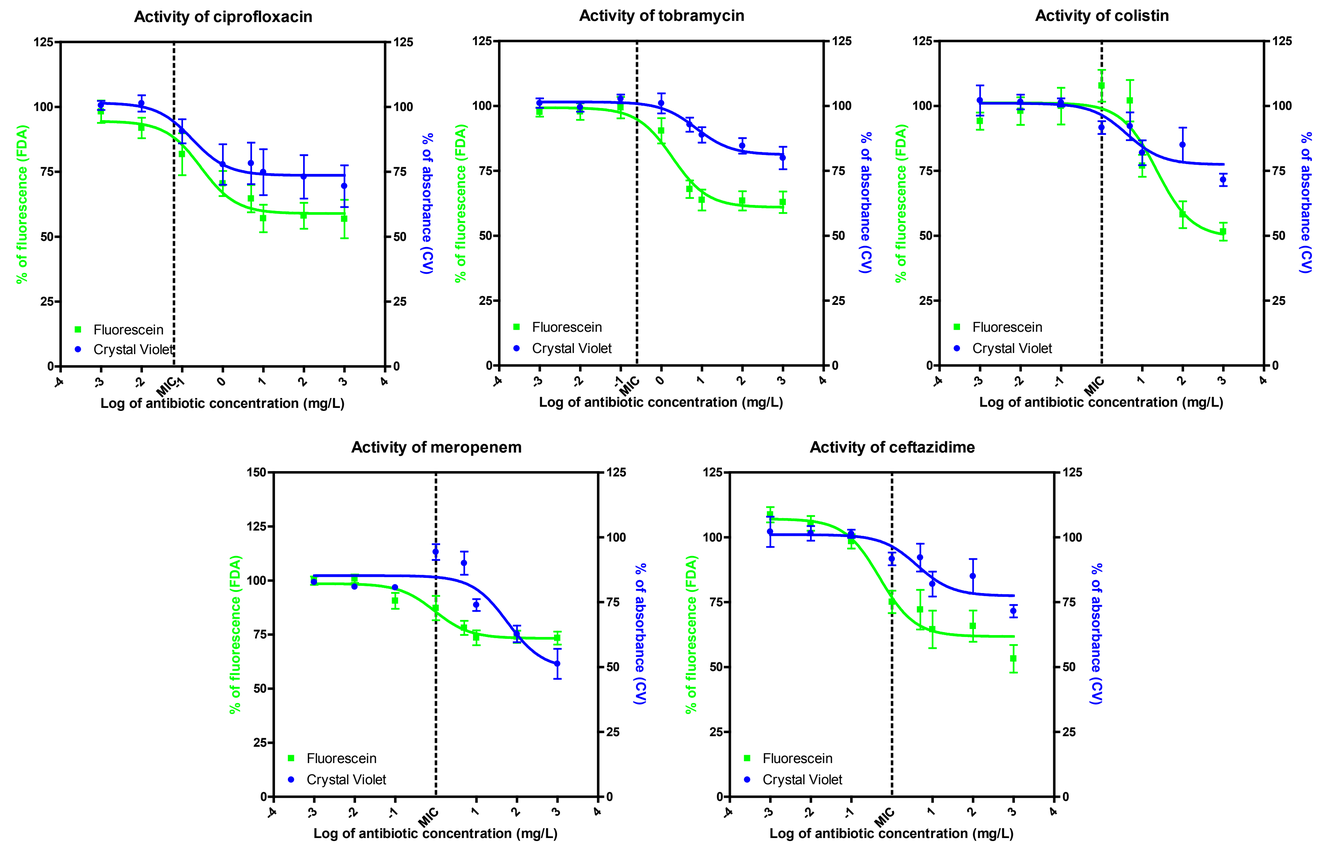
Results

A. Kinetic of development :



- Biomass and viability increased over time to reach a plateau on day 4 for both parameters.

B. Antibiotic activity on mature biofilms and pharmacodynamic parameters:



- Antibiotic activity was concentration-dependent towards both viability and biomass.
- **Pharmacodynamic parameters towards viability:**
  - E_{max} ranged from 26% (meropenem) to 51% (colistin) reduction *vs* untreated controls.
  - C_{25} ranged from 1 to 25 time the MIC.
- **Pharmacodynamic parameters towards viability:**
  - E_{max} ranged from 18% (tobramycin) to 41% (meropenem) reduction *vs.* untreated controls.
  - C_{25} were higher than 50 times the MIC.

antibiotic	MIC	E_{max}		C_{25}			
		Viability	Biomass	Viability		Biomass	
	µg/mL	%	%	µg/mL	x MIC	µg/mL	x MIC
Ciprofloxacin	0.06	41.07	26.39	0.35	5.85	3.38	56.30
Colistin	1	50.59	30.98	23.08	23.08	108.17	108.17
Ceftazidime	1	38.23	22.60	1.21	1.21	/	/
Meropenem	1	26.73	40.90	12.73	12.73	100.18	100.18
Tobramycin	0.25	39.31	18.79	3.44	13.76	/	/

Conclusion

- Using coated plates and renewing the medium every day allow pseudomonal biofilms to mature quickly in around 4 days.
- As expected, antibiotics were much less effective and potent against bacteria in biofilms than against planktonic bacteria.
- This model could be used for the screening of new anti-biofilm agents.

References

1. Tré-Hardy M. *et al.* Effect of antibiotic co-administration on young and mature biofilms of cystic fibrosis clinical isolates: the importance of the biofilm model. International Journal of Antimicrobial Agents (2009) 33:40-45. PubMed 18801647
2. Peeters E. *et al.* Comparison of multiple methods for quantification of microbial biofilms grown in microtiter plates. Journal of Microbiological Methods (2008) 72:157-165 PubMed 18155789

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