

In-vitro model of *Pseudomonas aeruginosa* pulmonary biofilm to evaluate the efficacy of cationic antibiotics



Rana Awad¹, Sandrine Marchand^{1,2}, William Couet^{1,2}, Mohamad Nasser⁴, Frederic Tewes¹

¹ INSERM U1070, Université de Poitiers, France

² Service de Pharmacocinétique, CHU de Poitiers, France

³ PRASE, Université Libanaise, Hadath-Beirut

rana.awad@univ-poitiers.fr – 05.49.36.69.36



Introduction

- ❖ *Pseudomonas aeruginosa* (PA) develop as biofilms in chronic pulmonary infections in patients with cystic fibrosis¹
- ❖ Biofilms are aggregates of PA (50-100 µm) entrapped in a self-produced matrix of anionic polymers (alginate, DNA...), often surrounded by patient mucus²
- ❖ The activity of cationic antibiotics "ATB" such as tobramycin (TOB) and colistin (COL) against these biofilms is low *in-vivo* because of their interactions with the matrix

Purpose

- ❖ Develop an *in-vitro* model composed of anionic polymers found *in-vivo* in pulmonary PA biofilms to evaluate the efficacy of inhaled ATB used to treat chronic pulmonary infections

Material & Methods

- A bioluminescent strain of PA (PAOI-LUXCDEBA) was incorporated into large calcium alginate beads (∅ = 1200 µm).^{2,3} These beads were dispersed in artificial sputum medium⁴ (ASM) to produce an *in-vitro* PA pulmonary biofilm model
- The effectiveness of ATB (TOB and COL) was tested on the *in vitro* PA pulmonary biofilm by measuring :
 - PA bioluminescence kinetics during 40H
 - Bacterial concentrations (log₁₀ CFU / ml) after 40 h of exposure to ATB. These values were plotted as a function of ATB concentrations and modelled using the following inhibitor Emax model.⁵

$$CFU(t) = CFU_0 \times \left(1 - \frac{C^y}{C_{50}^y + C^y}\right)$$

CFU₀: CFU in the absence of ATB

C : concentration of ATB X MIC

C₅₀: [ATB] needed to achieve 50% of CFU₀

y: Hill factor

- ✓ The index with the best fit and low value of C₅₀ is more effective
- The development of resistance to these ATBs among surviving PAs was evaluated by measuring the MIC

Results

Evaluate the efficacy of ATB on the *in-vitro* model of PA pulmonary biofilm

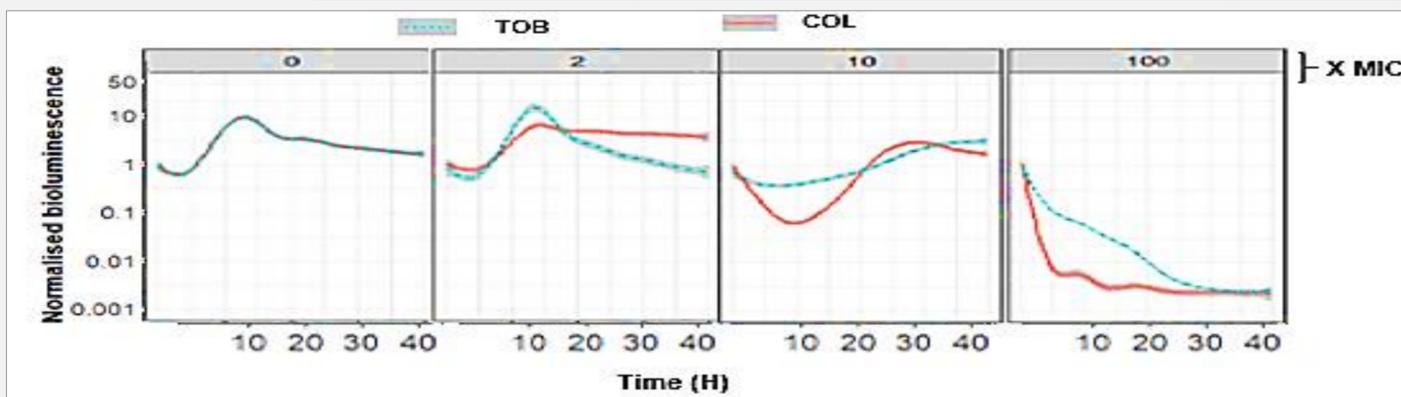


Fig.1: Bioluminescence kinetics of PA trapped in calcium alginate beads dispersed in ASM exposed to various COL or TOB concentrations times MIC (*MIC_{TOB} = 0.5 µg/ml, *MIC_{COL} = 1 µg/ml)

- Above 10 times the MIC, the decrease in bioluminescence was greater and faster with COL compared to TOB

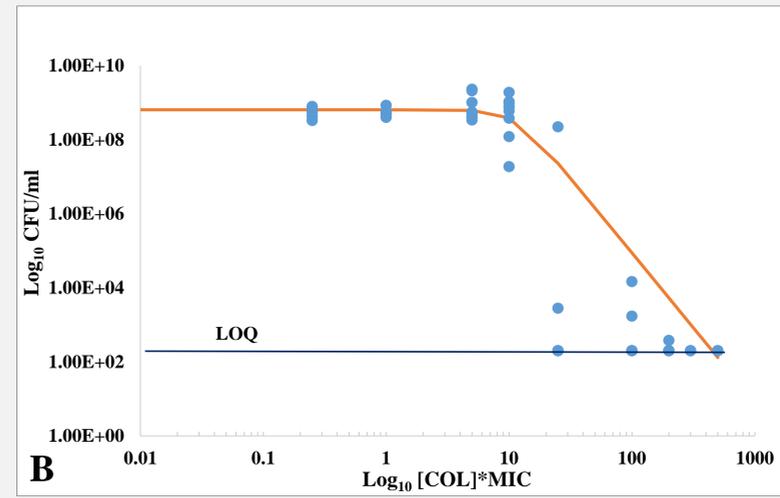
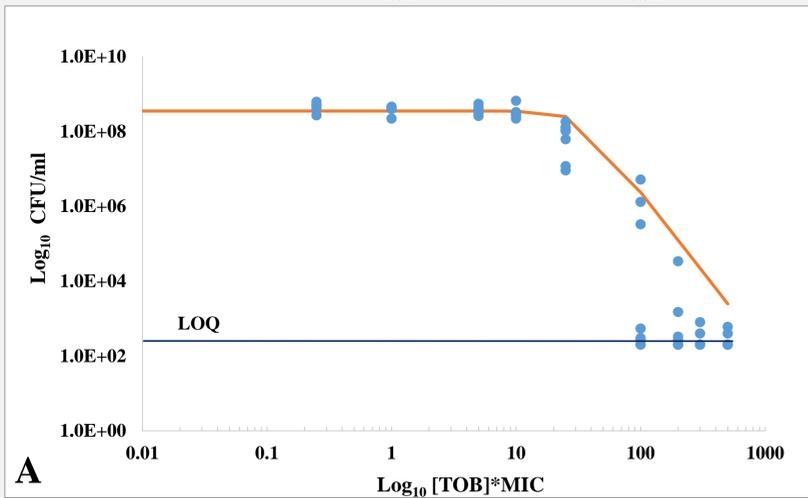


Table 1 : Parameter estimates for the Emax model plotted with different ATB tested on the *in vitro* model of PA pulmonary biofilm. Data were expressed as mean ± SD

Parameters	TOB	COL
CFU ₀ (PA colonies/ml)	3.52E+08	6.44E+08 ± 4.4E+07
C ₅₀ (µg/ml)	30.88 ± 3.26	11.07 ± 1.61
y	4.26 ± 0.18	4.04 ± 0.18

Fig. 2: PA concentrations loaded in calcium alginate beads dispersed in ASM after exposure during 40 hours to various TOB (A) or (COL) concentrations expressed in number of times the MIC (*LOQ: Limite of quantification = 200 CFU/ml). The estimated values of the Emax model parameters are shown in Table 1

- The C₅₀ (30.88 ± 3.26 µg / ml) obtained for the TOB was higher than that obtained for the COL (11.07 ± 1.61 µg / ml) suggesting a higher potency of the COL to reduce the bacterial burden in the PA pulmonary biofilm model
- After 40H of exposure to ATB, PA showed similar MIC value compared to the bacteria before being treated with these ATBs

Adaptation of PA to the presence of TOB

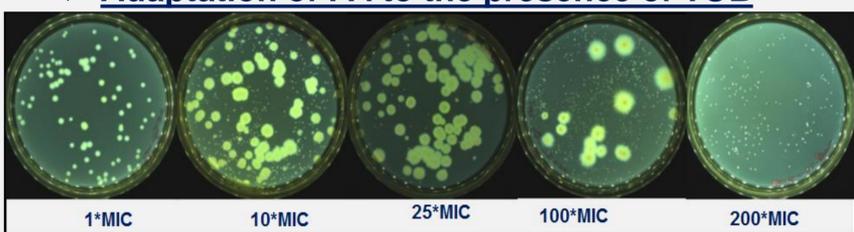


Fig. 3: Morphology of 72-hour PA colonies on Müller-Hinton agar obtained from PA extracted from the *in-vitro* pulmonary biofilm model exposed 40 h to TOB.

Two types of colonies were observed

- ✓ Normal and Small colony variant "SCV" (Described in CF patients with chronic pulmonary infections⁶)
- ✓ The bacteria of both types of colonies were bioluminescent
- ✓ SCV appeared only after 48 h of plating on Müller-Hinton agars
- ✓ SCV had similar MIC value compared to the MIC of bacteria before treatment with TOB
- ✓ Percentage of small colonies relative to total number of colonies increased with TOB concentration

Conclusion & Perspectives

- Bioluminescence measurements and colony counts show that COL was more effective than TOB on an *in-vitro* model of PA pulmonary biofilm, suggesting a potential better clinical efficacy of COL than TOB when treating these biofilms
- PA may persist in biofilms even when exposed to high concentration of TOB without developing resistance to this antibiotic

References

1. Hentzer M *et al*, J Bacteriol., 2001
2. Majken Sønderholm *et al*, npj Biofilms and Microbiomes vol 4, 2018
3. Pedersen S. *et al*. APMIS 1990
4. S.D. Dinesh, Artificial sputum medium, Protoc. exch, 2010
5. Elisabet I. Nelsen *et al*, Pharmacol Rev 2013
6. Richard A. Proctor *et al*, Nature Reviews Microbiology, 2006