Activity of antibiotics in combination with clarithromycin in an *in vitro* model of *Pseudomonas aeruginosa* mature biofilm in the context of lung infection in Cystic Fibrosis patients

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Introduction

Pseudomonas aeruginosa (*Pa*) is a major cause of respiratory tract infections in Cystic Fibrosis (CF) adult patients (1). These patients therefore require repetitive and prolonged antibiotic treatments with antipseudomonal drugs. Achieving high concentrations at the site of infection (lung) is highly crucial to eradicate the chronic biofilm infection. Beside antipseudomonal antibiotics, these patients often receive macrolides by oral route for their anti-inflammatory properties and their capacity to inhibit biofilm formation by inhibiting bacterial Quorum Sensing.

Aim of the study

Our aim was to determine whether macrolides can also cooperate with antibiotics against pre-formed, mature biofilms of *Pa*, as this better represents the clinical situation. We aimed to identify synergistic combinations between a clinically-relevant antipseudomonal drug from different classes and a macrolide (important for its anti-inflammatory properties) that could be potentially developed as a viable future CF treatment.

Methods

Biofilms of PAO1 (or PAO1-GFP) were grown in 96-well cell culture microplates (or on cover slips in 6-well plates for microscopy experiments) in cation-adjusted Mueller-Hinton broth (CA-MHB) or in Artificial Sputum medium (ASM) mimicking the composition of CF patients' sputum (2) using a starting inoculum of 1-2x10^6 CFU/mL. The plates were incubated at 37°C. Mature 4-day old biofilms were then exposed to tobramycin (aminoglycoside), ciprofloxacin, levofloxacin (fluoroquinolones), colistin (polymyxin), or meropenem (β-lactam) alone or in combination with clarithromycin (macrolide) over a wide range of concentrations during 24 h at 37°C. Total bacterial viability within biofilm was quantified by fluorescein diacetate assay (3). Confocal laser scanning microscopy images were obtained using Cell Observer Spinning Disk (Carl Zeiss AG) and were analyzed using AxioVision Microscopy Softrware (Carl Zeiss AG) to determine biofilm thickness. Synergy was defined as a significantly higher reduction in total bacterial viability or biofilm thickness for combinations vs antibiotic alone.

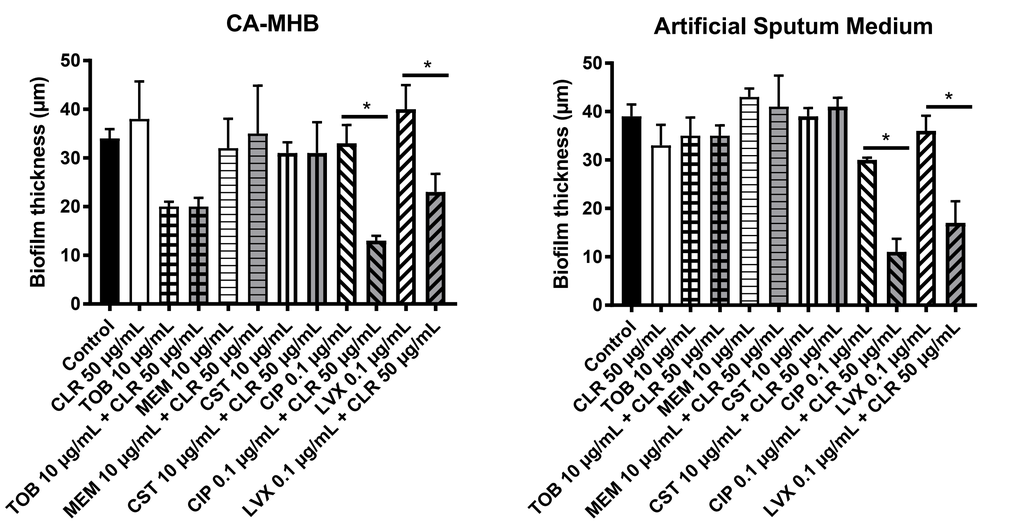


Figure 1: Biofilm thickness in µm as evaluated in Confocal Microscopy of 5-day old mature PAO1 biofilm after 24 hours of antibiotic exposure in CA-MHB and ASM.

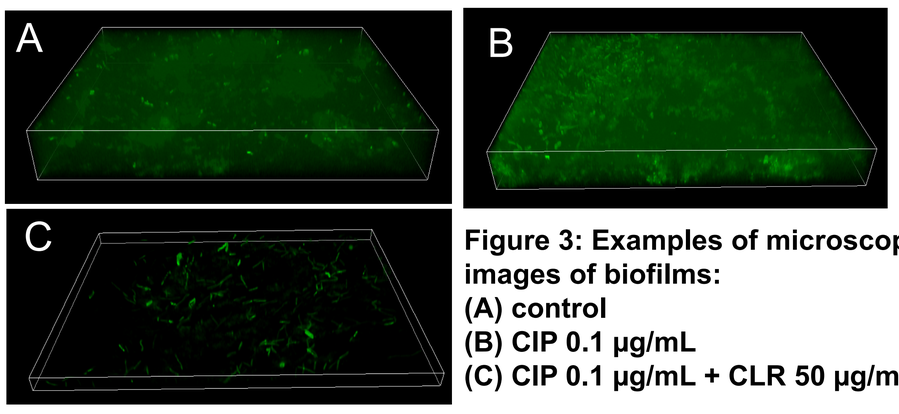
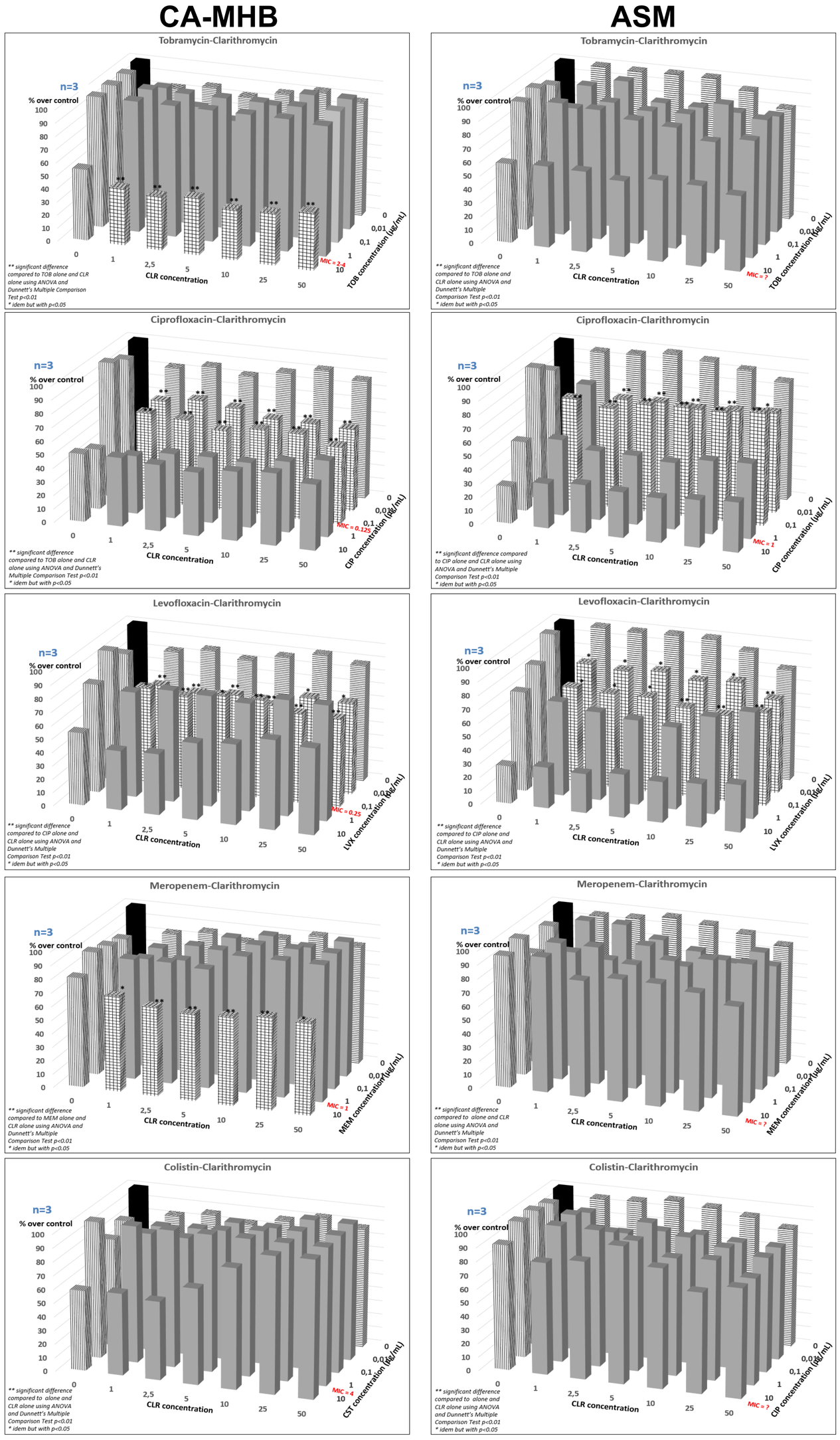


Figure 3: Examples of microscopy images of biofilms:
(A) control
(B) CIP 0.1 µg/mL
(C) CIP 0.1 µg/mL + CLR 50 µg/mL

Results

In CA-MHB, synergy was observed for combinations of clarithromycin with tobramycin (at 10 µg/mL, maximal reduction of 39%), ciprofloxacin (at 0.01 and 0.1 µg/mL, maximal reduction of 54%), levofloxacin (at 0.01 and 0.1 µg/mL, maximal reduction of 60%), and meropenem (at 10 µg/mL, maximal reduction of 65%), but not with colistin (Fig. 1). In ASM, all antibiotics were less active than in CA-MHB, except fluoroquinolones (ciprofloxacin, levofloxacin), which were also the only ones to maintain synergistic activity with clarithromycin (Fig. 1).

Figure 1: Activity of different antibiotics in combination with clarithromycin in CA-MHB (left) and ASM (right) as measured by total bacterial viability within the biofilm (FDA assay).



Considering biofilm thickness as observed in confocal microscopy, only fluoroquinolone-clarithromycin combinations showed synergy with significant thickness reduction compared to control and fluoroquinolones alone (Fig. 2 and 3). Globally, activity was also reduced in ASM vs CA-MHB.

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Conclusion

Globally, clarithromycin increases the antipseudomonal activity of all antibiotics except colistin against mature *Pa* biofilms. This synergy is maintained only for fluoroquinolones (both ciprofloxacin and levofloxacin) in ASM, probably due to a preferential interaction of the other drugs with mucus constituents (mucin, DNA, proteins) (4,5,6).

Combinations of fluoroquinolone-clarithromycin are also the only ones that showed synergy in reducing biofilm thickness in both media.

Taken together, this study highlights the interest of combining fluoroquinolones and clarithromycin (already useful alone as anti-inflammatory agent) as a potential CF treatment in the context of chronic respiratory infection by *Pa*. Further studies, however, are warranted to document the interest of this combination *in vivo*.