Liposome-Ciprofloxacin inhibits *Mycobacterium avium subsp hominissuis* (MAH) microaggregate formation in dose and time-related manner

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ABSTRACT

Introduction: MAH is an important pathogen in individuals with immunosuppression as well as with underlying lung pathology such as cystic fibrosis (CF), bronchiectasis (BE) and emphysema. In such patients, MAH causes further debilitation that may require long courses of therapy, frequently with serious side-effects and therapeutic failures. MAH itself may be the primary cause of BE. We evaluated whether liposomal ciprofloxacin (Ciprofloxacin for Inhalation, CFI, Lipoquin®) would affect the establishment of MAH microaggregates, which is the first step for biofilm formation in the lung airways. CFI is efficacious against *M. avium* and *M. abscessus* in biofilm and macrophage assays [Blanchard et al., ATS 2014:A6677] and in mouse lung infection models of *M. avium* [Bermudez et al., ATS 2015:A6293] and *M. abscessus* [Blanchard et al., ICAAC 2015:B-536]. CFI is delivered directly to the respiratory tract where it achieves very high concentrations while the systemic exposure by ciprofloxacin is very low. CFI has completed a Phase 2 clinical trial to manage lung infection due to *Pseudomonas aeruginosa* (PA) in patients with CF [Bruinenberg et al., RDD 2010:73]. Pulmaquin®, a mixture of CFI and unencapsulated ciprofloxacin, has completed a Phase 2 trial to manage non-CF BE patients chronically colonized with PA [Serisier et al., Thorax 2013; 68:812] and is now in Phase 3 for this indication.

Methods: MAH strains 104 and A5 are clinical isolates; both strains form robust biofilms *in vitro* and *in vivo*. HEp- 2 cells are oropharyngeal epithelial cells, cultured in presence of RPMI-1640 medium. Biofilm and microaggregates of MAH were developed both on plastic surface and on the surface of epithelial cells. CFI (15 or 300 µg ciprofloxacin/ml) was added at the same time as the bacteria, or 1h, 2h, 4h, 12h, 24h, or 48h following infection.

Results: While 15 µg/ml CFI significantly inhibited microaggregate formation (31%) when added simultaneously to the infection versus control, the 300 µg/ml CFI concentration was significantly inhibitory when added 1 h post infection versus control (53%) and versus ciprofloxacin (45%). For the epithelial cell monolayer model, CFI was significantly inhibitory even when added 4 h post infection following infection versus control (71%) and versus ciprofloxacin (75%). The formation of microaggregate *in vivo* is dependent on the expression of MAV_3013 and MAV_0831. CFI inhibited the expression of MAV_3013 and MAV_0831 when added to the cell monolayer at time 0.

Conclusion: CFI treatment at the time of infection at concentrations that may be achievable in the respiratory tract using inhalation of liposomal ciprofloxacin in humans can inhibit MAH microaggregate formation and prevent biofilm formation.

INTRODUCTION

MAH is an important pathogen in individuals with immunosuppression as well as with underlying lung pathology such as cystic fibrosis (CF), bronchiectasis (BE) and emphysema. In such patients, MAH causes further debilitation that may require long courses of therapy, frequently with serious side-effects and therapeutic failures. MAH itself may be the primary cause of BE. We evaluated whether liposomal ciprofloxacin (Ciprofloxacin for Inhalation, CFI, Lipoquin®) would affect the establishment of MAH microaggregates, which is the first step for biofilm formation in the lung airways. CFI is efficacious against *M. avium* and *M. abscessus* in biofilm and macrophage assays [Blanchard et al., ATS 2014:A6677] and in mouse lung infection models of *M. avium* [Bermudez et al., ATS 2015:A6293] and *M. abscessus* [Blanchard et al., ICAAC 2015:B-536]. CFI is delivered directly to the respiratory tract via inhalation where it achieves very high concentrations while the systemic exposure by ciprofloxacin is very low. CFI has completed a Phase 2 clinical trial to manage lung infection due to *Pseudomonas aeruginosa* (PA) in patients with CF and BE [Bruinenberg et al., RDD 2010:73; Bruinenberg et al, ATS 2010:A3192]. Pulmaquin®, a mixture of CFI and unencapsulated ciprofloxacin, has completed a Phase 2 trial to manage non-CF BE patients chronically colonized with PA [Serisier et al., Thorax 2013; 68:812] and is now in Phase 3 for this indication.

METHODS AND MATERIALS

- MAH strains 104 and A5 are clinical isolates; both strains form robust biofilms in vitro and in vivo.
- HEp- 2 cells are oropharyngeal epithelial cells, cultured in presence of RPMI-1640 medium.
- Biofilm and microaggregates of MAH were developed both on plastic surface and on the surface of epithelial cells.
- CFI or free ciprofloxacin (15 or 300 µg ciprofloxacin/ml) was added at the same time as the bacteria, or 1h, 2h, 4h, 12h, 24h, or 48h following infection.

RESULTS

Gene expression (qRT-PCR): MAV-3013 and MAV-0831, associated with microaggregate formation measured at t=1 h

Antibiotic added at t=0	Hsp65	MAV_3013	MAV_0831
No antibiotic	6.2 ± 0.3	8.4 ± 0.7	6.1 ± 0.4
CFI (15 µg/ml)	5.9 ± 0.8	3.3 ± 0.5	2.7 ± 0.6
Ciprofloxacin (15 µg/ml)	6.9 ± 0.4	7.9 ± 0.5	7.5 ± 0.3

RESULTS

Effect of Treatment of MAH Biofilm on a Plastic Surface (both MAC 104 and MAC A5)

15 µg/ml	Hrs	Control (CFU)	CFI (CFU)	Ciprofloxacin (CFU)
	0	1×10^6	$6.9 \pm 0.4 \times 10^5$ *	$8.5 \pm 0.3 \times 10^5$
	1	$1 \pm 0.2 \times 10^6$	$9.1 \pm 0.3 \times 10^5$	$1.2 \pm 0.3 \times 10^6$
	2	$1.3 \pm 0.6 \times 10^6$	$1.1 \pm 0.5 \times 10^6$	$1.4 \pm 0.5 \times 10^6$
	4	$1.4 \pm 0.5 \times 10^6$	$1.4 \pm 0.3 \times 10^6$	$1.6 \pm 0.3 \times 10^6$
	12	$2.3 \pm 0.4 \times 10^6$	$2.4 \pm 0.5 \times 10^6$	$2.8 \pm 0.4 \times 10^6$
	24	$4.1 \pm 0.2 \times 10^6$	$4.4 \pm 0.3 \times 10^6$	$4.9 \pm 0.3 \times 10^6$

*p<0.05 vs. Control

300 µg/ml	Hrs	Control (CFU)	CFI (CFU)	Ciprofloxacin (CFU)
	0	1×10^6	$1.6 \pm 0.3 \times 10^5$ *†	$7.3 \pm 0.5 \times 10^5$
	1	$1 \pm 0.2 \times 10^6$	$4.7 \pm 0.4 \times 10^5$ *†	$8.6 \pm 0.4 \times 10^5$
	2	$1.3 \pm 0.6 \times 10^6$	$7.9 \pm 0.3 \times 10^5$	$1.5 \pm 0.3 \times 10^6$
	4	$1.4 \pm 0.5 \times 10^6$	$1.5 \pm 0.4 \times 10^6$	$1.7 \pm 0.5 \times 10^6$
	12	$2.3 \pm 0.4 \times 10^6$	$2.5 \pm 0.5 \times 10^6$	$2.5 \pm 0.3 \times 10^6$
	24	$4.1 \pm 0.2 \times 10^6$	$4.7 \pm 0.3 \times 10^6$	$4.1 \pm 0.4 \times 10^6$

*p<0.05 vs. Control
†p<0.05 vs. Ciprofloxacin

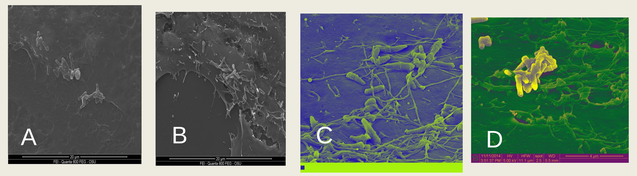
Effect of Treatment of MAH biofilm on an Epithelial Cell Monolayer (MAC 104)

300 µg/ml	Hrs	Control (CFU)	CFI (CFU)	Ciprofloxacin (CFU)
	0	$6.9 \pm 0.3 \times 10^6$	$8.1 \pm 0.5 \times 10^4$ *†	$3.8 \pm 0.3 \times 10^5$
	1	$7.3 \pm 0.4 \times 10^5$	$1.7 \pm 0.4 \times 10^5$ *†	$5.9 \pm 0.5 \times 10^5$
	2	$9.6 \pm 0.3 \times 10^5$	$5.5 \pm 0.5 \times 10^5$ *†	$9.1 \pm 0.4 \times 10^5$
	4	$2.1 \pm 0.3 \times 10^6$	$6.1 \pm 0.3 \times 10^5$ *†	$2.4 \pm 0.6 \times 10^6$
	12	$3.9 \pm 0.3 \times 10^6$	$3.9 \pm 0.6 \times 10^6$	$3.8 \pm 0.4 \times 10^6$
	24	$4.5 \pm 0.6 \times 10^6$	$4.6 \pm 0.3 \times 10^6$	$4.8 \pm 0.5 \times 10^6$

*p<0.05 vs. Control
†p<0.05 vs. Ciprofloxacin

RESULTS

Electron Micrographs of MAH Microaggregates



Microaggregates: 4 hr (A), 24 hr (B) and after exposure to CFI (C) and free ciprofloxacin (D)

Changes in Expression of MAV-3013 and MAV 0831 Biofilm (%) and Microaggregate (%): Antibiotic added concomitant to bacteria at t=0

(%)	Biofilm (%)	Microaggregate
No antibiotic	100 ± 2	100 ± 3
CFI (300 µg/ml)	30 ± 7	38 ± 12
Ciprofloxacin (300 µg/ml)	91 ± 4	95 ± 6

MIC of Ciprofloxacin and Liposomal Ciprofloxacin against the MAH Strains in Plankton

Antibiotic	MIC (104 and A5)
Ciprofloxacin	4-8 mcg/ml
CFI	4-8 mcg/ml

CONCLUSIONS

Liposomal ciprofloxacin (CFI) is as effective as ciprofloxacin to inhibit the growth of MAH in plankton but is much more effective to inhibit the formation of microaggregates of MAH that are the precursors of MAH biofilms.

ACKNOWLEGMENT

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