Supernatants of *Pseudomonas aeruginosa* incubated with azithromycin sub-MIC concentrations fail to induce cytokine production in A549 and THP-1 cell lines

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Introduction & purpose

There are numerous clinical reports of the beneficial effects of low-dose long-term azithromycin treatment in chronic inflammatory lung diseases which cannot be attributed solely to its antibacterial activity. This study was undertaken to test the effect of azithromycin sub-MIC concentrations on *Pseudomonas aeruginosa* and *P. aeruginosa* - host interaction.

Methods

All studies were performed with *Pseudomonas aeruginosa* strain isolated from cystic fibrosis patient. The MIC was determined according to CLSI guidelines, while MBEC and MBIC were determined using MBEC assay (Innovotech). Pyoverdine and pyocyanin levels were determined by measurement of fluorescence (Ex400/Em460) and absorbance (520 nm), respectively. Elastase activity in supernatants was assessed by measurement of absorbance (490 nm) after an overnight incubation with CongoRed (Sigma). CFU numbers were determined by plating serially diluted bacteria on the surface of nutrient agar plates.

To address the effect of azithromycin on *P. aeruginosa* interaction with the host, human lung epithelial (A549) and monocytic (THP-1) cell lines were stimulated with supernatants of bacteria grown overnight with or without the azithromycin in sub-MIC concentrations. Following 6 hours incubation, the concentration of pro-inflammatory cytokines, TNFα and IL-8, were determined by ELISA (R&D Systems).

Results

Antipseudomonal profile

Azithromycin had no clinically relevant antimicrobial activity against *Pseudomonas aeruginosa* (MIC=128 µg/mL) and no effect on formed biofilm (MBEC >256 µg/mL). However, in a biofilm formation assay, azithromycin displayed activity at concentrations lower than its MIC (MBIC=4 µg/mL) (Table 1).

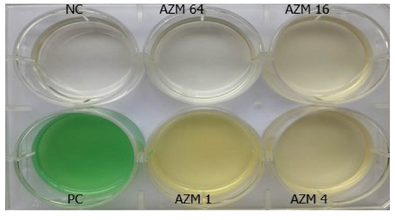
Table 1. Antimicrobial profile of azithromycin against *P. aeruginosa* in comparison to standard antibiotics

	P.aeruginosa			
Antibiotics	MIC	MBC	MBIC	MBEC
AZITHROMYCIN	128	>128	4	>128
PIPERACILLIN	4	16	4	>256
CEFTAZIDIME	2	2	2	>256
CIPROFLOXACIN	1	4	1	>256
MEROPENEM	0.5	0.5	0.5	>256
TOBRAMYCIN	1	1	1	256
COLISTIN	1	2	1	8
CLARITHROMYCIN	128	>128	128	>128

MIC – minimal inhibitory concentration; MBC – minimal bactericidal concentration; MBIC – minimal biofilm inhibition concentration; MBEC – minimal biofilm eradication concentrations; all values are expressed in µg/mL.

Virulence factor production

Virulence factors production was inhibited at concentrations below the MIC and below the concentration that affected the CFU number (16 µg/mL). Pyoverdine (PYOV) production was inhibited at >1 µg/mL and pyocyanin (PYOC) and elastase (ELAST) at concentrations >0.25 µg/mL (Figure 1.).



NC – negative control, media without bacteria
PC – positive control, media with bacteria
AZM 64 – media with bacteria in presence of 64 µg/mL of azithromycin
AZM 16 – media with bacteria in presence of 16 µg/mL of azithromycin
AZM 4 – media with bacteria in presence of 4 µg/mL of azithromycin
AZM 1 – media with bacteria in presence of 1 µg/mL of azithromycin

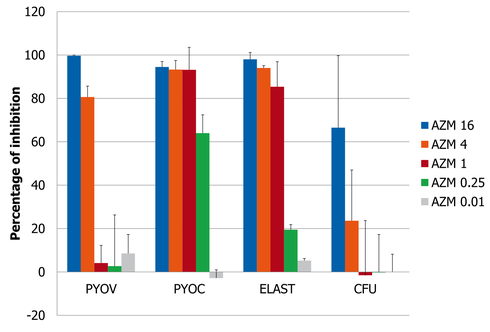


Figure 1. Azithromycin at sub-MIC concentrations inhibits virulence factors production

Inflammatory profile

Pseudomonas aeruginosa supernatants stimulated TNFα and IL-8 production in THP-1 and A549 cell lines. Supernatants of bacteria grown in the presence of sub-MIC concentrations of azithromycin were significantly weaker inducers of cytokine production.

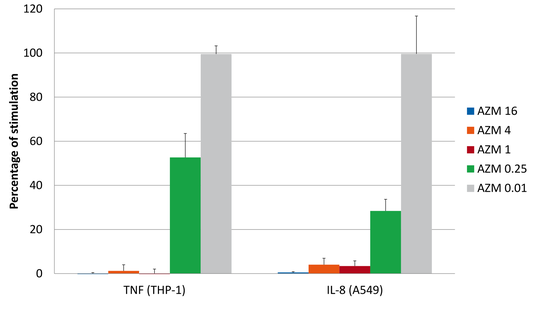


Figure 2. *P.aeruginosa* supernatants in presence of AZM sub-MIC concentrations fail to iduce cytokine production

Conclusions

Azithromycin at sub-MIC concentrations reduces the production of *Pseudomonas aeruginosa* virulence factors and decreases the pro-inflammatory potential of the *P. aeruginosa* culture supernatant. Observed effective concentrations are far below concentrations reported in the literature to be active in numerous *in vitro* anti-inflammatory assays and are much more in line with the concentrations present in humans. Therefore, these data indicate additional beneficial effects of azithromycin treatment of chronic inflammatory diseases with *P. aeruginosa* colonization.

References

Bosnar M. et al., *J Pharmacol Exp Ther*, 2009; 331:104-113

Fernandez-Olmos A. et al., *Int J Antimicrobial Agents*, 2012; 40:173-176

Meyer M. et al., *Am J Respir Cell Mol Biol*, 2009; 41:590-602

Pena J. et al., *Infect Immun*, 2009; 77:2857-2865

Vrancic M. et al., *British J Pharmacol*, 2012; 165:1348-1360

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