

P0143

Poster Session I

Basic science: pathogenesis

SUPERNATANTS OF *P.AERUGINOSA* INCUBATED WITH AZITHROMYCIN SUB-MIC CONCENTRATIONS FAIL TO INDUCE CYTOKINE PRODUCTION IN A549 AND THP-1 CELL LINES

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Objectives:

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 *P.aeruginosa* and *P.aeruginosa* host interaction.

Methods:

All studies were performed with *P.aeruginosa* isolated from cystic fibrosis. 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 agar plating serial diluted bacteria.

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

Results:

Azithromycin had no clinically relevant antimicrobial activity against *P.aeruginosa* (MIC=128 μ g/ml) and no effect on formed biofilm (MBEC >256 μ g/ml). However, in a biofilm formation assay, azithromycin displayed activity at concentrations lower than its MIC (MBIC=4 μ g/ml). In addition, virulence factors production was inhibited at concentrations that were below the MIC and below the CFU affecting concentration (16 μ g/ml). Pyoverdine production was inhibited at >1 μ g/ml and pyocyanin and elastase at concentrations >0.25 μ g/ml.

P.aeruginosa supernatants stimulated TNF α and IL-8 production in THP-1 and A549 cell lines, respectively. Supernatants grown in the presence of sub-MIC concentrations of azithromycin were significantly weaker inducers of cytokine production. The effect was still detectable at 0.25 μ g/ml (512 times lower than the MIC value). These effects cannot be attributed to direct anti-inflammatory effects of the drug since no inhibition of cytokine production was observed in A549 and THP-1 cells stimulated with *P.aeruginosa* supernatants treated with corresponding concentrations of azithromycin.

Conclusion:

Azithromycin in sub-MIC concentrations reduces *P.aeruginosa* virulence factors production and decreases the pro-inflammatory potential of the *P.aeruginosa* culture supernatant. Observed effective concentrations are far below concentrations reported in 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.