Azithromycin reduces the production of virulence factors in *Pseudomonas aeruginosa* by inhibiting quorum sensing

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**Introduction**

The outcome of diffuse pan-bronchiolitis (DPB) is greatly affected by lung superinfection with *P. aeruginosa* while prolonged administration of low doses of 14- and 15-membered macrolides significantly augments life expectancy of these patients. Macrolides may act via effects on the immune system to modify the inflammatory response to the disease and the infection as reviewed in this *Journal*. In agreement with this possibility the efficacy of erythromycin in DPB patients was observed independently of *P. aeruginosa*. On the hand macrolides have been shown to display a variety of effects against *P. aeruginosa*, which may contribute to the their efficacy in DPB. The MIC of macrolides for most *P. aeruginosa* is in the range 128-512 mg/L, and judged by conventional criteria, these bacteria are classified as resistant to macrolides. However, on agar containing clinically achievable concentrations of azithromycin or 14-membered macrolides viability of *P. aeruginosa* was significantly reduced after 48 h of incubation, but not after shorter periods. In addition, *P. aeruginosa* accumulated azithromycin intracellularly over a period of 12 to 36 h.

**Macrolides interfere with virulence in *P. aeruginosa***.

The macrolides have shown to inhibit the expression of several virulence factors including exotoxin A, proteases, elastase, phospholipase C, DNase, lecithinase, gelatinase, lipase, pyocyanin and motility. Other studies showed that sub-inhibitory concentrations of macrolides interfered with the production and the stability of alginate and biofilm in *P. aeruginosa*. Since these products and effects are associated with the development of *P. aeruginosa* infections, it may be advanced that besides anti-inflammatory effects, macrolides may also exert anti-pseudomonas effects.

**Quorum sensing as a regulator of virulence factors.**

In *P. aeruginosa*, the production of exoproteins acting as virulence factors is affected by the population size. Each individual bacterial cell is able to sense other members in the same population and in response, expresses specific sets of genes, including those encoding several virulence factors (Fig. 1). This cell-to-cell communication is called quorum sensing. In *P. aeruginosa*, the las (for elastase) and rhl (for rhamnolipids) quorum sensing systems regulate the production of several extracellular virulence factors, including elastase and rhamnolipids. Each system is composed of a gene *lasR* or *rhlR*, and a gene encoding an autoinducer synthetase, *lasI* or *rhlI* (Fig. 1). Autoinducer synthetases *lasI* and *rhlI* are required for the synthesis of autoinducer molecules 3-oxo-C12-homoserine lactone (12-HSL) and C6-homoserine lactone (4-HSL) respectively. Autoinducers form complexes 12-HSL-LasR and 4-HSL-RhlR,

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which act as transcriptional activators of several target genes encoding exo-proteins, including molecules acting as virulence factors. They also diffuse freely (or easily) in the vicinity of the bacterial cells, so that when cell density achieve a certain threshold, the concentration of autoinducers is sufficient for acting in other cells of the population (cell-to-cell signalling). Other metabolic pathways, which are not fully identified, associates quorum sensing with the production of alginate and biofilm.

Azithromycin inhibits quorum sensing in *P. aeruginosa*. Since macrodilides interfere with exo-proteins associated with the quorum sensing, as well as the production of biofilm also affected by quorum sensing, the possibility that macrodilides could interfere with the quorum sensing circuitry was considered. All experiments below were performed at the onset of stationary phase (when quorum sensing is active) at macrodilides concentrations where bacterial growth was not notably affected. Azithromycin was chosen because preliminary experiments showed that was the most active among five other macrodilides on a weight basis. At a concentration of 2 mg/L, i.e. a level clinically achievable, the inhibitory effect of azithromycin on elastase and rhamnolipids production was confirmed. A subsequent set of experiments, using -lacZ reporter fusions placed in appropriate plasmids and β-galactosidase assays, showed that azithromycin reduced significantly the transcription of different quorum sensing genes: *rhlA*, *rhlR*, *lasR*, *lasI* and *rhlI*. In particular azithromycin, 2mg/L, reduced the transcription of *lasI* and *rhlI* by 80% and 50%, respectively. In good agreement with this observation, specific bioassays indicated that the concentrations of the corresponding autoinducers 12-HSL and 4-HSL decreased by 94 and 72%, respectively, in presence of the macrodilides. Since the expression of *lasR* and *rhlR* genes is dependant on the presence of adequate autoinducer levels, their transcription was measured in presence of exogenous 12-HSL and 4-HSL at concentrations of 10 μM. As a result, the expression of *rhlR* was completely restored, and that of *lasR* almost completely restored. Complementation with the autoinducers also restored most of the production of elastase in the expression of *rhlAB*.

The general conclusion of these series of experiments was that azithromycin interferes with the synthesis of autoinducers, leading to a reduction of virulence factor production.

**Clinical potential of quorum-sensing inhibition by azithromycin.**

In addition to its anti-inflammatory effect, azithromycin clearly inhibits the quorum sensing circuitry of *P. aeruginosa*. Even not tested already, 14-membered macrolides are likely to share the same potential, since these molecules have been shown to decrease the production of quorum-sensing-regulated exo-proteins as discussed below. Most of the quorum-sensing-regulated virulence factors cause tissue damage. In addition the 12-HSL autoinducer exhibits immunomodulatory activity and stimulates the
production of interleukin-8 by respiratory epithelial cells\textsuperscript{14}. Altogether all these observations suggest that administration of 14- and 15-membered macrolides to patients suffering from chronic lung infection by \textit{P. aeruginosa}. This potential may contribute to the improvements in lung functions documented in patients suffering from DPB or cystic fibrosis\textsuperscript{15} with \textit{P. aeruginosa} infections. In addition to these relatively rare conditions, macrolides may also prove useful in preventing and treating ventilator associated \textit{P. aeruginosa} pneumonia.

\textbf{REFERENCES}


