The putative de-N-acetylase DnpA increases intracellular and biofilm-associated persistence upon fluoroquinolone exposure in Pseudomonas aeruginosa

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Introduction & Purpose
Persisters are antibiotic-treated bacteria that are transiently refractory to antibiotic killing (1). They are associated with dormant lifestyles and cause treatment failures. The putative de-N-acetylase DnpA (unknown substrate) has been shown to increase persister levels in P. aeruginosa exposed to fluoroquinolones in broth (2). This study assesses the possible role of DnpA in the poor efficacy of antibiotics against P. aeruginosa in two models of persistent infections (intracellular infection & biofilms).

Materials & Methods
Bacterial strains: PAO1 and its ΔdnpA deletion mutant (1). Extracellular persistence assay: bacteria exposed to antibiotics at 50xMIC for 5 h; persister fraction calculated as the ratio of cfu for antibiotic-exposed bacteria to controls (2). Intracellular activity: 24 h incubation of infected human THP-1 monocytes with antibiotics (0.001-200 mg/L) to obtain a full activity curve (2). Activity against biofilms: 24 h incubation of biofilms with antibiotics (same conc. range); residual viability assessed by Fonds de la Recherche Scientifique

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Results

1. Persisters in broth

2. Intracellular activity of antibiotics

3. Antibiotic effect on biofilm formation and preformed biofilm

4. Influence of antibiotics on gene expression

Conclusions
DnpA contributes to persistence of P. aeruginosa exposed to ciprofloxacin intracellularly or in biofilms. The underlying mechanism could involve the overexpression of the fluoroquinolone target. Inhibiting DnpA is an attractive strategy to improve fluoroquinolone activity in persistent infections.

REFERENCES