Antimetabolite drugs as inhibitors of Pseudomonas aeruginosa biofilm growth and virulence: potential chemotherapics and tools in target identification for new antimicrobials

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Background. In cystic fibrosis (CF) patients, Pseudomonas aeruginosa-induced pneumonia requires frequent antibiotic treatment. Low sensitivity to many antibiotics by P. aeruginosa has prompted the search for novel drugs aimed at the specific inhibition of pathogenesis-related processes. Antimetabolite drugs, such as the pyrimidine analogue flucytosine (FC), are effective inhibitors of biofilm formation and of virulence factors' production, and show promising activity against P. aeruginosa infections in animal models. However, their utilization in therapy is hindered by possible toxicity, occurrence of resistance, and by lack of precise information of their mechanism of action.

Hypothesis and objectives. The main hypothesis behind this work was that antimetabolite drugs other than FC could share its properties and affect P. aeruginosa virulence and persistence in the host. In addition, we wanted to establish the specific mode of action of FC, as a starting point towards the development of new drugs sharing the same mechanism of action and showing lower risks of long term toxicity.

Essential Methods. We tested antimetabolite drugs for their antimicrobial activity on both PAO1 and PA14 strains of P. aeruginosa. Potential antivirulence activity was tested as inhibition of pyoverdine production. To understand FC mode of action we performed a screening using a genome library to identify genes conferring resistance to FC activity.

Results. Unlike FC, other antimetabolite drugs and inhibitors of nucleotide biosynthesis failed to inhibit pyoverdine production, despite showing some antibiofilm activity, with the only exception of fluorouridine, a similar compound to FC. This result suggests that pyoverdine inhibition by FC is mediated by a mechanism of action unique to this molecule and not shared with other antimetabolites. To identify FC molecular target, we expressed a Salmonella genome library in Escherichia coli, in which FC also strongly inhibits expression of specific genes. We found that FC effects were reversed by expression of RNase E, an essential protein involved in RNA turnover.

Spin-off for research & clinical purposes. Our results suggest that RNase E might be the target of FC. Thus, it is possible that inhibitors of RNase E can be identified with similar antivirulence effect with higher efficacy in vivo and lower risk of long term toxicity.

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References

1. ImperiF, Massai F, Facchini M, Frangipani E, Visaggio D, Leoni L, Bragonzi A,ViscaP. Repurposing the antimycotic drug flucytosine for suppression of Pseudomonas aeruginosa pathogenicity. Proc Natl Acad Sci U S A 2013. 110:7458-7463. https://doi.org/10.1073/pnas.1222706110 PMid:23569238 PMCid:PMC3645532

2. Kirienko DR, Revtovich AV, Kirienko NV. A high-content, phenotypic screen identifies fluorouridine as an inhibitor of pyoverdine biosynthesis and Pseudomonas aeruginosa virulence. mSphere 2016. 1:4.

3. Ueda A, Attila C, Whiteley M, Wood TK. Uracil influences quorum sensing and biofilm formation in Pseudomonas aeruginosa and fluorouracil is an antagonist. Microbial Biotech 2009. 2: 62-74. https://doi.org/10.1111/j.1751-7915.2008.00060.x PMid:21261882 PMCid:PMC3815422

4. AntonianiD, Rossi E, Rinaldo S, Bocci P, Lolicato

M, Paiardini A, Raffaelli N, Cutruzzolà F,Landini P. The immunosuppressive drug azathioprine inhibits biosynthesis of the bacterial signal molecule cyclic-di-GMP by interfering with intracellular nucleotide pool availability. Appl Microbiol Biotechnol 2013. 97:7325-7336. https://doi.org/10.1007/s00253-013-4875-0
PMid:23584245