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Novel EptA inhibitors increase the susceptibility of Neisseria gonorrhoeae to killing by innate immune defenses

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Background

Antibiotics are the primary treatment for gonococcal (Ng) infections; however, the emergence of multidrug-resistance (MDR) has rendered almost all classes of antibiotics ineffective. Lipooligosaccharide phosphoethanolamine (PEA) transferase A, EptA, is responsible for the decoration of PEA to lipid A in the pathogenic Neisseria. The PEA-decorated lipid A is essential for bacterial resistance to killing by innate immune defenses such as cationic antimicrobial peptides (CAMPs). We have identified a novel class of small molecules, INH-2, that inhibit EptA with improved potency for further testing in models of disease.

Aim/Methods

INH-2 is a synthetic derivative of compound [137] with improved potency towards Ng which was identified using established pipelines. INH-2 was tested for improved potency in assays for sensitivity to CAMP LL-37 using antimicrobial susceptible and MDR-isolates WHO P, G, and X. INH-2 was tested for step-wise evolution of resistance to CAMP, polymyxin B, over 60 passages. The ability of INH-2 to clear infected cell lines (Hela and RAW cell lines) was tested in the presence and absence of a stimulator of CAMP expression, HDACi (histone deacetylase inhibitor).

Results

Exposure of WT-Ng to INH-2 restored sensitivity to killing by 6.4-3.2μM LL-37 which was the same MIC as the isogenic ΔeptA mutant in all strains tested. Post-treatment of infected cell lines with INH-2 resulted in the reduction of WT-Ng load to the same degree as the isogenic ΔeptA mutant of each isolate. INH-2 exhibited no toxicity towards ΔeptA mutant in any assay. HDACi treatment was used to stimulate CAMP expression from the cell lines which was measured in the supernatant by bacterial killing. HDACi treatment of the post-infection model with INH-2 resulted in >98% reduction in bacterial load which was sustained for at least 12 hrs.

Conclusions

INH-2 represents a novel class of EptA inhibitors which is not affected by the expression of the multidrug efflux pump MtrCDE. It showed low potential for resistance development over 60 passages. HDACi was used to stimulate CAMPs from the cell lines which resulted in clearance of these models of infection. Future work will assess the utility of INH-2 in female mouse models of uncomplicated gonorrhoea.