Background and Aims

Growth

P. aeruginosa (Pa), an opportunistic pathogen, is a leading agent of hospital-acquired infections and chronic infections in patients with cystic fibrosis, causing mortality and morbidity [1, 2]. Those infections are hard to eradicate as Pa is intrinsically resistant to many antibiotics, largely due to low permeability of outer membrane [3], which impairs the efficacy of antibiotics. In addition, Pa encounters in vacuoles where it resides in acidic pH of the vacuoles hosting bacteria in infected cells. The combination of rifampicin and polymyxin B is highly synergistic, it encounters in vacuoles. In these conditions, the strong synergy was observed when combined with rifampicin, a disused antibiotic for which Pa resistance is low, against intracellular forms of infection.

We are currently working to explore the generality of this observation — the combination results (black circles) are connected with lines to the zero interaction surface, (2) lines and regions of surface are colored blue for synergies and red for antagonisms. Certain combinations of polymyxin B and rifampicin are synergistic in acidic conditions. Circles on figures 1-4 stand for change in CFUs after 24h of antibiotic treatment from an initial inoculum of 10^6 CFU/mL. Red circles stand for the results of rifampicin and yellow for polymyxin monotherapy. From the results of monotherapies, the expected outcome was calculated for the combinations of the two drugs assuming there is no drug-drug interaction (grey surface). The result of experiment outcome of combinations is recorded as black figures. For better visualization: (1) the combination results (black circles) are connected with lines to the zero interaction surface, (2) lines and regions of surface are colored blue for synergies and red for antagonisms.

P. aeruginosa (Pa) causes recalcitrant infections [1, 2] that are hard to treat due to its low permeability to antibiotics [3] and its hiding in biofilms [4] and inside host cells [5-6]. Polymyxins work by disrupting bacterial membranes of Gram-negative bacteria and, in doing so, are the antipseudomonal antibiotics of choice for polymyxin B (PMB) in combination with rifampicin (RIF), a disused antibiotic for which Pa resistance is low, against intracellular forms of infection.

Our aim was to examine the potential of polymyxin B (PMB) in combination with rifampicin (RIF), a disused antibiotic for which Pa resistance is low, against intracellular forms of infection.

Methods


2. MIC determination: microdilution (CLSI recommendations) with successfully assessed according to the EUCAST interpretive criteria (http://www.eucast.org).

3. Antibiotic activity against intracellular bacteria: (i) phagocytosis of bacteria by human THP-1 monocytes: (a) elimination of non-internalized bacteria by exposure to gentamicin; (ii) incubation with a wide range of extracellular antibiotic concentrations (0.003-100 x MIC) of RIF and PMB to 24 h to obtain full bacterial killing and concentration-response curves; (iii) Intracellular activity was evaluated at 24 h as the difference in CFUs in THP-1 (drug) units from the initial inoculum. Concentration-response curves were fitted with four-parameter logistic regression (Hill-Langmuir equation) fixing the slope at 1.

4. Antibiotic activity against bacteria in broth: overnight culture of bacteria was diluted to 0.5 x 10^6 CFU/mL in fresh cation adjusted Mueller-Hinton broth with 100 mM MES at pH 5.5.

5. Combination of polymyxin B and rifampin results in two orders of magnitude better killing than expected

6. Polymyxin B and rifampicin against P. aeruginosa ATCC27853 (in broth at neutral pH)

7. Polymyxin B and rifampicin against P. aeruginosa ATCC27853 in broth at neutral pH

8. Polymyxin B and rifampicin against P. aeruginosa ATCC27853 in broth at pH 5.5

9. In vitro synergistic combinations of polymyxin B and rifampicin

10. Broth pH 5.5 ± 0.1

11. Broth pH 7.4 ± 0.1

12. Results

13. Conclusion

14. Acknowledgments and Funding

15. References

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