Limited Maximal Activity Without Marked Loss of Potency of Antibiotics against Intracellular Forms of Staphylococcus aureus and Pseudomonas aeruginosa: An Analysis with Bactericidal Antibiotics from Different Pharmacological Classes in a Pharmacodynamic Model of Human THP-1 Infected Monocytes

Françoise Van Bambeke and Paul M. Tulkens

Pharmacologie cellulaire et moléculaire, Louvain Drug Research Institute, Université catholique de Louvain, Bruxelles, Belgium

Background, Model and Data Retrieval

Infections caused by Staphylococcus aureus and Pseudomonas aeruginosa remain a therapeutic challenge, due, in part, to the ability of these organisms to survive in intracellular compartments of eukaryotic cells. In this context, our laboratory has built a pharmacodynamic model allowing for a quantitative assessment of their concentration-dependent effects in these environments.

In brief, S. aureus or P. aeruginosa isolates (susceptible to the antibiotics studied) are either (i) placed in broth, or (ii) phagocytized by THP-1 monocytes. Antibiotics are then added to the monocytes culture medium for 24 h at concentrations ranging from 0.01 to 100 x MIC to the MBC to obtain full concentration-dependent responses and to calculate two pertinent pharmacodynamic parameters based on Hill equations (sigmoidal function): (i) E_max (relative potency) extracellular concentration resulting in no apparent bacterial growth from CFU at 0 x MIC (CFU at 0 x post phagocytosis inoculum)

Typical Results

Data obtained with various strains of S. aureus and P. aeruginosa (PA103) for different pharmacological classes (see Results) shown to be known to be bactericidal (>3 log10 CFU decrease in 24h) in broth were retrieved from our publications and used to calculate, on a homogenous fashion, the corresponding antibiotic relative potencies (Cs) and the maximal relative activities (Emax) towards intracellular vs extracellular bacteria (see references).

Discussion and Conclusions

Discussion

• All antibiotics tested showed a sharp decrease of the intracellular maximal relative efficacy (E_max) compared to broth, and in most cases, are not bactericidal in cells (less than 3 log3 CFU decrease compared to the post-phagocytosis inoculum) even though all are bactericidal in broth.

• As the calculation of E_max assumes an infinitely large extracellular concentrations of antibiotics, the loss of efficacy shown here cannot be due to a simple global lack of diffusion and availability of the antibiotics. Conversely, since all antibiotic tested show similar relative potencies (Cs), in the concentration range of their MIC in broth, antibiotics are able to penetrate cells and reach their target (at least in part).

• We also showed (not illustrated here) that bacteria remaining in cells show the same MIC as the original inoculum when collected and grown again in broth and are not Small Colony variants.

• Intracellular bacteria, for a low but significant proportion of the original inoculum, are probably in a state of non-responsive to antibiotics. This may explain why S. aureus and P. aeruginosa in intracellular inocula are more likely to prove difficult to treat, as intracellular bacteria remaining viable in cells may reinfect ion once antibiotic therapy acting on their extracellular forms is discontinued.

Background, Model and Data Retrieval

Infections caused by Staphylococcus aureus and Pseudomonas aeruginosa remain a therapeutic challenge, due, in part, to the ability of these organisms to survive in intracellular compartments of eukaryotic cells. In this context, our laboratory has built a pharmacodynamic model allowing for a quantitative assessment of their concentration-dependent effects in these environments.

In brief, S. aureus or P. aeruginosa isolates (susceptible to the antibiotics studied) are either (i) placed in broth, or (ii) phagocytized by THP-1 monocytes. Antibiotics are then added to the monocytes culture medium for 24 h at concentrations ranging from 0.01 to 100 x MIC to the MBC to obtain full concentration-dependent responses and to calculate two pertinent pharmacodynamic parameters based on Hill equations (sigmoidal function): (i) E_max (relative potency) extracellular concentration resulting in no apparent bacterial growth from CFU at 0 x MIC (CFU at 0 x post phagocytosis inoculum)

Typical Results

Data obtained with various strains of S. aureus and P. aeruginosa (PA103) for different pharmacological classes (see Results) shown to be known to be bactericidal (>3 log10 CFU decrease in 24h) in broth were retrieved from our publications and used to calculate, on a homogenous fashion, the corresponding antibiotic relative potencies (Cs) and the maximal relative activities (Emax) towards intracellular vs extracellular bacteria (see references).

Discussion and Conclusions

Discussion

• All antibiotics tested showed a sharp decrease of the intracellular maximal relative efficacy (E_max) compared to broth, and in most cases, are not bactericidal in cells (less than 3 log3 CFU decrease compared to the post-phagocytosis inoculum) even though all are bactericidal in broth.

• As the calculation of E_max assumes an infinitely large extracellular concentrations of antibiotics, the loss of efficacy shown here cannot be due to a simple global lack of diffusion and availability of the antibiotics. Conversely, since all antibiotic tested show similar relative potencies (Cs), in the concentration range of their MIC in broth, antibiotics are able to penetrate cells and reach their target (at least in part).

• We also showed (not illustrated here) that bacteria remaining in cells show the same MIC as the original inoculum when collected and grown again in broth and are not Small Colony variants.

• Intracellular bacteria, for a low but significant proportion of the original inoculum, are probably in a state of non-responsive to antibiotics. This may explain why S. aureus and P. aeruginosa in intracellular inocula are more likely to prove difficult to treat, as intracellular bacteria remaining viable in cells may reinfect ion once antibiotic therapy acting on their extracellular forms is discontinued.

References

Data were retrieved from the following published works (but only selected data are illustrated). DOI:10.1016/j.ijantimicag.2011.03.002

F.V.B. is Senior Research Associate of the Belgian F.W.O. (Fonds pour la Recherche Scientifique) and of the Fonds de la Recherche Scientifique Médicale (F.R.S.-F.M.S.) (Belgium). Françoise Van Bambeke and Paul M. Tulkens are supported by a grant from the F.R.S.-F.W.O. (Fonds pour la Recherche Scientifique - Fonds Wetenschappelijk Onderzoek). These Institutions and Organizations had no role in the collection and interpretation of the data or the preparation of the manuscript. DOI:10.1016/j.ijantimicag.2011.03.002