A New Approach to Predict Anti-staphylococcal Effects of Antibiotic Combinations Using *in Vitro* Dynamic Models

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Background

- Data obtained using checkerboard techniques do not always predict the activity of antibiotic combinations, possibly because the “optimal” concentration ratio of the combined agents might not correspond to clinically attainable ratios of the respective 24-hour areas under the concentration – time curve (AUCs).
- To explore if susceptibility testing at antibiotic concentration ratios equal to the AUC ratios used in pharmacodynamic studies is more predictive of bacterial killing, *Staphylococcus aureus* was exposed to linezolid (LZD) and rifampicin (RIF) in an *in vitro* dynamic model that simulates single and combined treatments at therapeutic and sub-therapeutic ratios of AUC_{LZD} and AUC_{RIF}.

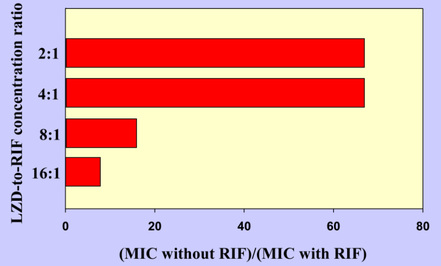
Materials/Methods

- Susceptibility of a clinical isolate of *S. aureus* to LZD and RIF was tested for the single agents and their combinations at the therapeutic LZD-to-RIF concentration ratio (4:1; AUC_{LZD} 240 μg×h/ml to AUC_{RIF} 60 μg×h/ml) and sub-therapeutic concentration ratios (2:1; AUC_{LZD} 120 μg×h/ml to AUC_{RIF} 60 μg×h/ml), 8:1 (AUC_{LZD} 120 μg×h/ml to AUC_{RIF} 15 μg×h/ml or AUC_{LZD} 240 μg×h/ml to AUC_{RIF} 30 μg×h/ml) and 16:1 (AUC_{LZD} 240 μg×h/ml to AUC_{RIF} 15 μg×h/ml). Susceptibility testing was performed in triplicate by using broth microdilution techniques according to CLSI methods [1].
- Using the above AUC_{LZD} and AUC_{RIF} (single drug treatments) and AUC_{LZD} to AUC_{RIF} ratios (combination treatments), twice daily dosing of LZD with a half-life of 6 h [2] and once-daily dosing RIF with a half-life of 3 h [3] given alone and in combination were simulated for 5 consecutive days.
- A previously described dynamic model [4] was used in simulations of single drug treatments with linezolid and rifampicin. To simulate combination treatments, the model was modified according to the Blaser and Zinner principle [5] to provide simultaneous mono-exponential elimination of linezolid and rifampicin.
- The central compartment of the model was sampled daily for viable counts and antibiotic concentrations from the beginning of simulated treatments to 120 h. LZD and RIF concentrations were determined by a validated HPLC method.

Results

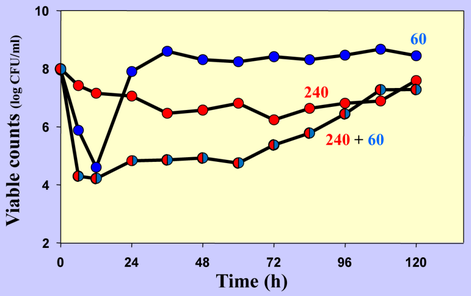
- RIF enhanced susceptibility of *S. aureus* to LZD in a concentration-dependent manner. At LZD-to-RIF concentration ratios of 2:1 and 4:1 the MIC of LZD dropped 67 times compared to the MIC determined without RIF (2 μg/ml); at the concentration ratios of 8:1 and 16:1, i.e. at a smaller proportion of RIF in the combinations, – 16 and 8 times, respectively (Fig. 1).
- Unlike RIF, LZD did not influence RIF susceptibility of *S. aureus*: MICs of RIF determined with and without LZD were similar (0.008-0.016 μg/ml).

Fig. 1.* Effect of RIF on LZD susceptibility of *S. aureus



- In simulations of multiple-dose pharmacokinetics of LZD-RIF combinations, killing of *S. aureus* was more rapid and intensive compared with single treatments – see for example, time courses of viable counts using the therapeutic AUC_{LZD} and AUC_{RIF} (Fig. 2), possibly as a result of lowering the MIC of LZD and thereby increasing the actual AUC/MIC ratios.

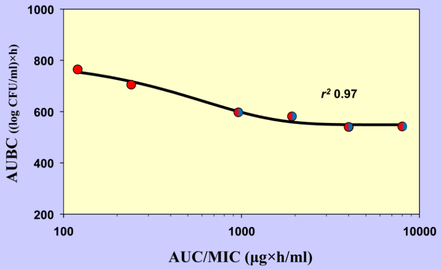
***Fig. 2.* Time courses of *S. aureus* exposed to LZD (●) and RIF (●), alone and in combination (●)**



AUCs of LZD and RIF (in μg×h/ml) are indicated by the numbers at each curve

- Indirectly, this is confirmed by the observation that the areas under time-kill curve (AUBCs) determined in combined and single treatments could be plotted against AUC/MIC expressed as LZD concentrations on the same sigmoid graph (r^2 0.97) (Fig. 3).

***Fig. 3.* AUC/MIC relationships with AUBC observed in single treatments with LZD (●) and combined treatments with LZD and RIF (●)**



Conclusion

These findings suggest that the antibacterial effects of LZD-RIF combinations can be predicted by AUC/MICs of LZD using its MIC determined at pharmacokinetically-derived LZD-to-RIF concentration ratios.

References:

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