

IN-VITRO PHARMACODYNAMICS OF TR-700, THE ACTIVE MOIETY OF PRODRUG TR-701, A NOVEL OXAZOLIDINONE, IN A KILL-CURVE MODEL

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Abstract

Background: The aim of this study was to determine the effectiveness of TR-700, the active form of its prodrug TR-701, a novel oxazolidinone, against three strains of methicillin-resistant *Staphylococcus aureus* (MRSA), that are also resistant to linezolid. **Methods:** Three strains of *S. aureus* (CM/05, ATCC 33591, NRS 271) were studied in this experiment. The minimum inhibitory concentrations (MIC) for TR-700 were determined according to the microbroth dilution method as standardized by CLSI to be 0.5, 0.25 and 4 µg/mL respectively. Cultures of each strain were grown in Mueller-Hinton broth (MHB) at static concentrations of 0, 0.25, 0.5, 1, 2, 4, 8, 16 times their MIC. Serial dilutions of samples taken every 2 hours were made, plated on sheep blood agar (SBA) and allowed to incubate for 20 hours at 37° C. A modified Emax model was fit to the kill curve data and allowed to estimate EC50 values for each strain. For *S. aureus* NRS 271 an additional Hill factor of 1.4 was included.

Results: TR-700 showed bacteriostatic activity against all three strains with maximum reductions of 0.18 log, 0.28 log, and 1.73 log for *S. aureus* CM/05, *S. aureus* ATCC 33591 and *S. aureus* NRS 271 respectively. The estimated EC50 values for TR-700 were found to be 0.14, 0.07 and 1.32 µg/mL respectively.

Conclusion: The estimated EC50 values correlate well with previously determined MIC values (r²=0.99). The described PK/PD model will allow integration of these pharmacodynamic properties of TR-700 with its pharmacokinetic profile to facilitate dose selection for future clinical trials.

Introduction

Instead of using the MIC only as a static parameter for pharmacodynamic behavior, a kill-curve experiment was done to assess the in vitro activity of TR-700, a novel oxazolidinone. This approach allows to follow time and concentration dependent behavior of an antibiotic drug.

Results of this experiment can be described by a mathematical model, which can then be used to model activity of an antibiotic at physiologically achievable concentrations that were derived from clinical trials.



Materials and Methods

Bacterial strains: Three strains of clinically relevant methicillin-resistant *Staphylococcus aureus* (*S. aureus* ATCC 33591, NRS 271, CM/05) were used for the experiment.

Minimal inhibitory concentrations (MIC): MICs for the three used *S. aureus* strains were attained using the CLSI standardized microdilution method. Briefly, a small volume of bacterial inoculum (106 CFU/mL) was added to microwells containing increasing concentrations of TR-700. The microtiter plate was then incubated at 37° C for 20 hours in ambient air. The MIC was read as the lowest concentration that showed no visible growth of bacteria. Resulting MICs can be read in Table 1.

Kill-curves: For this experiment an inoculum of 20 mL (106 CFU/mL) was prepared in tissue culture flasks. The inoculum was incubated at 37° C for 2 hours to allow bacteria to enter the log growth phase. After that, time-point zero samples were taken and antibiotic was added at concentration levels of 0, 0.25, 0.5, 1, 2, 4, 8 and 16 x MIC. Every two hours for 24 hours samples were taken from the flasks, serially diluted in saline solution and plated on Sheep blood agar (SBA). SBA plates were incubated for 20 hours at 37° C and ambient air.

Modelling: Bacterial counts obtained from the kill curves were fitted simultaneously to the following mathematical model using Scientist® 3.0 software (Micromath®, Saint Louis, MO):

$$\frac{dN}{dt} = \left[k_s \left(1 - \frac{N}{N_{max}} \right) - \left(\frac{E_{max} * c}{EC_{50} + c} \right) (1 - \exp^{-dk}) \right] * N$$

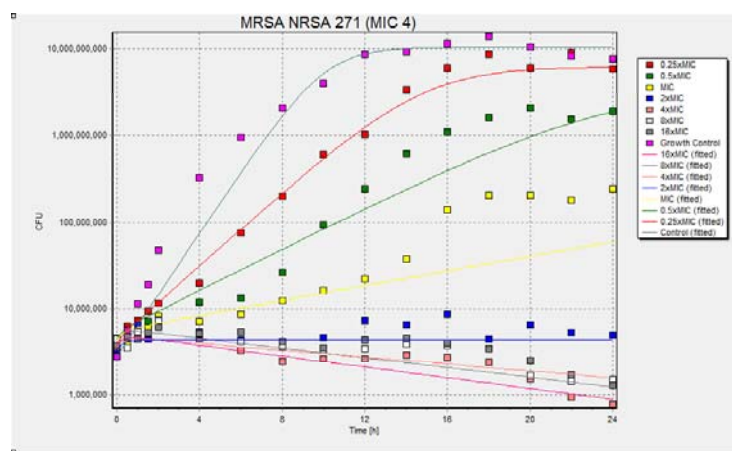
Where k_s is the bacterial growth rate constant, N and N_{max} are the bacterial and maximum achievable bacterial number, respectively and dk as a factor of describing delay in onset of kill.

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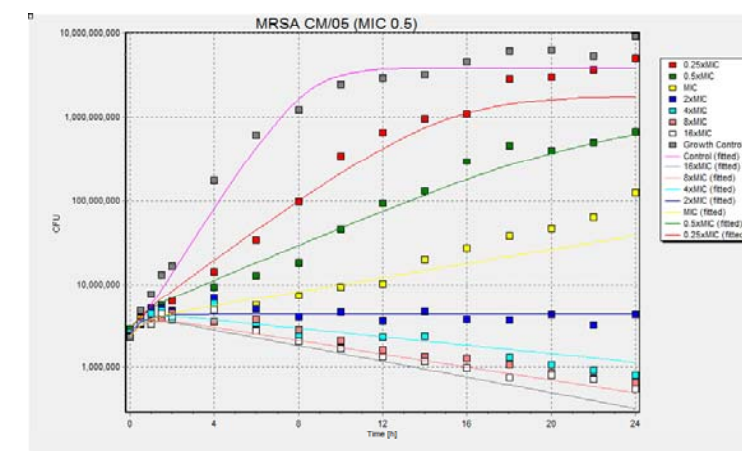
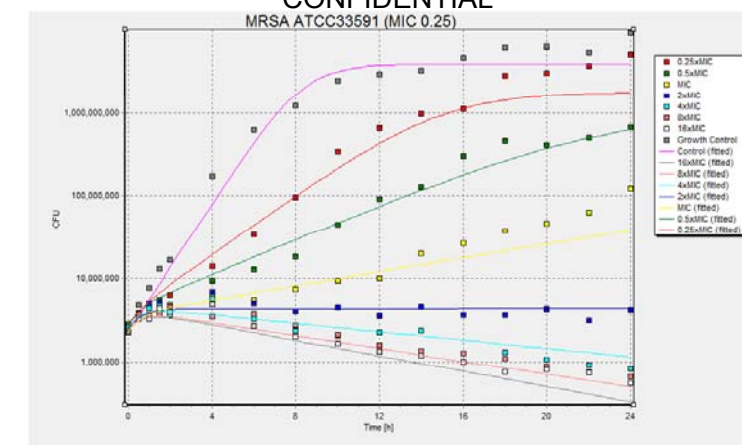
Results and Discussion

TR-700 showed bacteriostatic activity against all three *S. aureus* strains in the investigated time period of 24 hours. Overall reductions in bacterial count were 0.1 log, 0.28 log and 1.73 log for *S. aureus* CM/05, ATCC 33591 and NRS 271, respectively. MICs determined in this experiment correlate well with MICs reported in other publications. [1] The reported MIC values also correlate well with the estimated EC50 values.

Strain/ Parameter	<i>S. aureus</i> ATCC 33591	<i>S. aureus</i> NRS 271	<i>S. aureus</i> CM/05
EC ₅₀ [µg/mL]	0.07	1.32	0.14
MIC [µg/mL]	0.25	4	0.5
k _s [h ⁻¹]	0.89	0.82	0.89
N _{max} [CFU/mL]	3.85*10 ⁹	1.04*10 ¹⁰	3.85*10 ⁹
E _{max} [h ⁻¹]	1.02	0.9	1.02
Dk [h ⁻¹]	1.65	3.04	1.65
h	1	1.4	1



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Conclusion

It was shown, that the model could describe growth and kill characteristics of TR-700 in vitro. It will be useful for describing antibiotic behavior of TR-700 using actual drug concentrations obtained in clinical trials, thus making the process of dose decisions for future trials easier and more cost effective.