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Paper Poster Session

PK/PD of agents against Gram-positives

**Testing the mutant selection window hypothesis in *Staphylococcus aureus* resistance studies with linezolid using a mixture of antibiotic-susceptible cells and resistant mutants in an *in vitro* dynamic model**

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**Background:** *In vitro* resistance studies with bacteria that exhibit low mutation frequencies often fail because of the lack of spontaneous resistant mutants (RMs) in the starting inoculum. To test the mutant selection window (MSW) hypothesis as applied to linezolid-exposed *S. aureus*, a mixed inoculum of linezolid-susceptible and -resistant cells was used.

**Material/methods:** To obtain RMs, a clinical isolate of *S. aureus* 479 (MIC of linezolid 2 mg/L) was serially passaged in linezolid-containing Mueller-Hinton broth. The mutant prevention concentrations (MPCs) were determined for linezolid-susceptible cells ( $10^{10}$  CFU/ml) with RM isolated after the eighth passage (RM8;  $10^2$  and  $10^4$  CFU/ml) and without RM8. To provide linezolid concentrations within and out of the MSW, five-day treatments with twice daily linezolid were simulated over a 32-fold range of the ratio of area under the concentration – time curve (AUC) to the MIC in an *in vitro* model. The amplification of resistant mutants was monitored by plating on media with 2×, 4×, 8×MIC of linezolid. Time courses of resistant mutants were characterized by the area under the bacterial mutant concentration – time curve (AUBC<sub>M</sub>).

**Results:** RMs were enriched starting with the 7th-8th passage, with continued loss in susceptibility up to the 15th passage. MICs of linezolid determined for the isolated RMs were 2-, 4-, 8- and 16-fold higher than for the parental strain. The MICs for RMs remained constant after 20 passages on antibiotic-free medium. MPCs determined for the parental strain of *S. aureus* 479 with RM8 ( $10^2$  but not  $10^4$  CFU/ml) and without RM8 were similar (12 mg/L). Therefore, an inoculum containing  $10^{10}$  CFU per 100 ml central unit ( $10^8$  CFU/ml susceptible cells) and 1 ml of bacterial suspension of RM8 ( $10^2$  CFU/ml), i.e., one linezolid-resistant cell per  $10^8$  CFU of susceptible cells, was exposed to linezolid in *in vitro* model studies. *S. aureus* mutants resistant to 2×MIC of linezolid were enriched in a concentration-dependent pattern: little or no enrichment at the low (7.5 and 15 h) and high (120 and 240 h) AUC/MIC ratios in contrast to pronounced enrichment at the intermediate AUC/MICs (30 and 60 h). The enrichment of mutants resistant to 4× and 8×MIC of linezolid was less pronounced and occurred only at AUC/MICs 30 and 60 h. AUC/MIC-AUBC<sub>M</sub> curves were bell-shaped in concordance with the MSW hypothesis.

**Conclusions:** These findings support the MSW hypothesis as applied to linezolid-exposed *S. aureus*.