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CHARACTERIZATION OF THE NOVEL OXAZOLIDINONE TOREZOLID (TR-700) AND LINEZOLID SPONTANEOUS MUTATION FREQUENCIES AND RESISTANCE MECHANISMS IN *STAPHYLOCOCCUS AUREUS*

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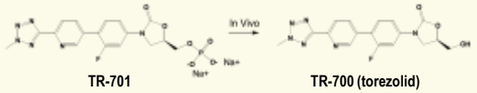
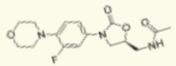
INTRODUCTION AND PURPOSE

- TR-701 is a novel oxazolidinone prodrug that has completed Phase 2 clinical trials for complicated skin and soft tissue infections
- TR-701 is rapidly converted to TR-700 (torezolid, the active moiety of TR-701) in vivo



TR-701 TR-700 (torezolid)

- Linezolid (LZD) served as a comparator agent in these studies



LZD

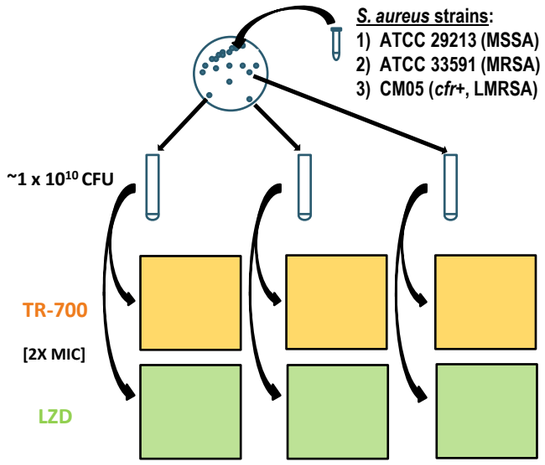
- Objectives:
  a) Compare the spontaneous mutation frequencies of 3 *S. aureus* strains under selection with TR-700 and LZD
  b) Investigate cross resistance trends
  c) Elucidate underlying resistance mechanisms

METHODS

- *S. aureus* strains ATCC 29213 (MSSA), ATCC 33591 (MRSA), and CM05 [*cfr*+, LZD-resistant MRSA (LMRSA)] were cultured at 37°C on Mueller-Hinton II agar (MHA) or in liquid broth (MHB)
- Spontaneous mutation frequencies were determined through plating of ~1 x 10^{10} CFU on large-format (245 x 245 mm) MHA plates containing 2X MIC of TR-700 or LZD
- MIC values were determined (CLSI) for a subset of TR-700 and LZD-selected mutants
- PCR was used to amplify individual *rrn* alleles and *rplC*, *rplD*, and *rplV* genes encoding ribosomal proteins L3, L4, and L22, respectively
- The domain V region of each 23S rRNA gene and entire *rplC*, *rplD*, and *rplV* genes were sequenced to elucidate underlying resistance mechanisms

RESULTS

I. Large format plating layout



a) Calculate mutation frequencies
b) Determine MICs
c) Elucidate resistance mechanisms

II. Spontaneous mutation frequencies

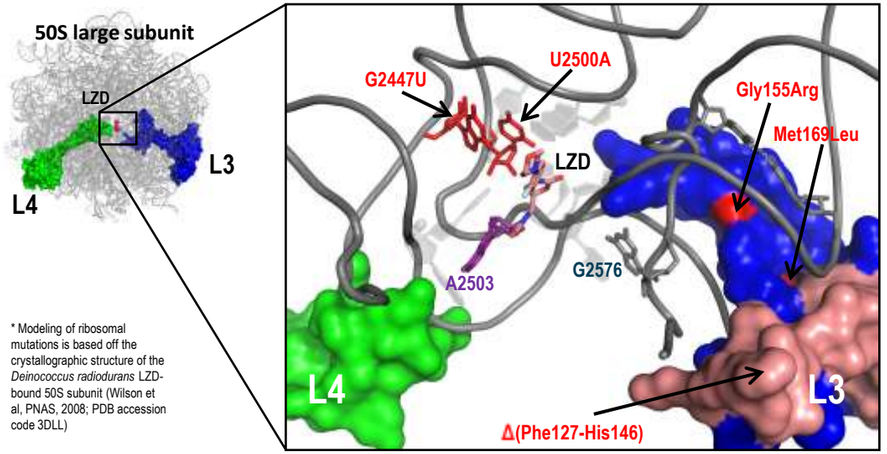
Strain	TR-700	LZD	LZD:TR
ATCC 29213 (MSSA)	$1.2x10^{-10}$	$1.9x10^{-9}$	16
ATCC 33591 (MRSA)	$2.0x10^{-10}$	$3.3x10^{-9}$	17
CM05 (*cfr*+, LMRSA)	$<4.8x10^{-11}$	$<4.8x10^{-11}$	n/a

III. Characteristics of wild-type and isogenic spontaneous mutant strains

		23S rRNA mutations			ribosomal protein mutations		MIC (µg/mL)			
strain	selection	rDNA	gene #(s)	prop.	*rplC*	L3	TR-700	LZD	TIA	VAN
ATCC 29213	wild-type	-	-	-	-	-	0.5	2	1	1
	LZD	G2447T	4	1/6	-	-	0.5	4	1	1
	TR-700 & LZD	T2500A	4	1/6	-	-	1	4	1	1
	TR-700	T2500A	4, 5	2/6	-	-	2	8	2	1
	TR-700 & LZD	-	-	-	G463C/A505T	Gly155Arg/Met169Leu	2	8	4	2
	TR-700	-	-	-	Δ(A384-A443)	Δ(Phe127-His146)	2	8	4	2
ATCC 33591	wild-type	-	-	-	-	-	0.25	1	0.5	1
	TR-700 & LZD	-	-	-	G463C	Gly155Arg	0.5	2	4	2
CM05	wild-type	-	-	-	-	-	0.5	8	nd	1

- The genetic basis of 23S rRNA mutations (rDNA), the specific mutant 23S alleles (gene #s), and the proportion (prop.) of mutant alleles over the total number of 23S alleles are given
- Ribosomal protein L3 mutations are shown for the gene (*rplC*) and protein (L3)
- MICs were performed for all strains against TR-700, linezolid (LZD), tiamulin (TIA), and vancomycin (VAN)

IV. Structural modeling of spontaneous ribosomal mutations



* Modeling of ribosomal mutations is based off the crystallographic structure of the *Deinococcus radiodurans* LZD-bound 50S subunit (Wilson et al, PNAS, 2008; PDB accession code 3DLL)

- Spontaneous ribosomal mutations (red) all cluster around the LZD binding site in the peptidyl transferase center of the 50S large subunit
- Bases A2503 (site of Cfr methylation) and G2576 (most common base mutated in LZD-resistant strains) are shown for reference

RESULTS

- 16-fold lower frequency of spontaneous mutations for TR-700 vs LZD
- TR-700 maintained a 4-8-fold potency advantage over LZD against all mutants, including those with novel mutations in ribosomal protein L3
- No spontaneous mutants were selected for LMRSA CM05 (*cfr*+)
- T2500A was the only 23S rRNA mutation identified through selection with TR-700
- 23S rRNA mutations conferring resistance to LZD included T2500A and G2447T
- TR-700 and LZD selected spontaneous mutations were also identified in ribosomal protein L3 including Gly155Arg and Gly155Arg/Met169Leu
- Selection with TR-700 identified a mutant with a 20 amino acid deletion of Phe127-His146 in L3
- Cross resistance between TR-700 and LZD was observed for all 23S rRNA and L3 mutations
- Cross resistance was observed between oxazolidinones and tiamulin for all L3 mutations

CONCLUSIONS

- Spontaneous mutation frequencies for *S. aureus* against TR-700 were >10-fold lower than for LZD
- TR-700 maintained a 4-8-fold potency advantage against LZD for all mutant strains, including those with novel L3 mutations and mutations unique to selection by TR-700

ACKNOWLEDGEMENTS

- We thank Dr. John Quinn for the *cfr*+ CM05 strain