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Activity of the novel FabI inhibitor Debio 1452 against intracellular forms of susceptible and resistant *S. aureus*: comparison with linezolid, vancomycin and daptomycin

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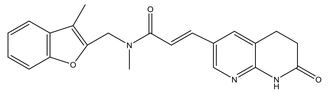
Background and Aims

Staphylococcus aureus remains a therapeutic challenge, due in part to the ability of this organism to acquire resistance mechanisms to most recommended antibiotics (1) and to survive in intracellular compartments of eukaryotic cells (2). In this context, it is therefore essential (i) to foster the discovery and development of novel antibiotics with mode(s) of action distinct from those in current use, and (ii) to assess the activity of these molecules against intracellular *S. aureus*.

Debio 1452 (formerly AFN 1252; see structure in Figure 1) is the active moiety of the prodrug Debio 1450, an IV and oral "first in class" antibiotic currently in Phase 2 clinical development for severe staphylococcal infections. It specifically targets staphylococcus species through inhibition of the staphylococcal FabI enoyl-Acyl carrier protein (ACP) reductase that catalyzes the last step in the elongation process of the fatty acid chain in these bacteria (3). Debio 1452, converted from Debio 1450 *in vivo,* displays excellent and selective potency against staphylococcal species, including methicillin-susceptible (MSSA) and methicillin-resistant (MRSA) clinical *S. aureus* isolates from diverse origins.

The goal of our study was to compare the intrinsic activity (MIC) and the intracellular activity of the active moiety of Debio 1450 (Debio 1452) with that of other anti-staphylococcal agents against a series of strains with different resistance phenotypes.

Figure 1: structure and main biophysical properties of Debio 1452



- pK_a=3.6 (uncharged [>94%] at pH 5 to 10
- Calculated log P and $logD_{pH7}$: 1.99 to 3.01 and 1.88 to 3.0
- Maximal water solubility at pH 5 to 7: 36 mg/L

Sources:
- PubChem (http://pubchem.ncbi.nlm.nih.gov/);
- SciFinder (https://scifinder.cas.org/scifinder);
- ChemBioDraw (http://www.cambridgesoft.com/)
- Reaxys® Reed Elsevier (http://www.reaxys.com)

Methods

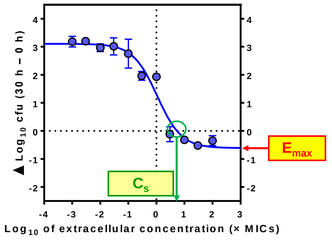
Bacterial strains and MIC measurements

- *S. aureus* reference strain ATCC 25923 (MSSA) and resistant strains NRS119, MU50 and SA040 LZD^R were obtained as indicated in the Table and grown in MHB as previously described (4).
- MICs were determined according to CLSI recommendations (4) and interpreted using available EUCAST clinical breakpoints (5).

Intracellular activity

- Experiments were performed with human THP-1 monocytes, displaying macrophage-like activity (6).
- Phagocytosis of opsonised bacteria was allowed for 1 h using a 4:1 bacteria-macrophage ratio, followed by elimination of extracellular bacteria by 45 min exposure to gentamicin (50 mg/L) and addition of the antibiotic (at extracellular concentrations varying from at least 1/100 to 100x the MIC to obtain full dose-response.
- Intracellular activity is expressed as the change in the initial inoculum at 30 h compared to the post-phagocytosis value (time 0).
- Data are used to fit a Hill equation allowing to determine the two key pharmacological descriptors of antibiotic activity (C_s and E_{max}) as described in Figure 2.

Figure 2: Pharmacodynamic model used in this study: analysis of the data



Pharmacodynamic parameters

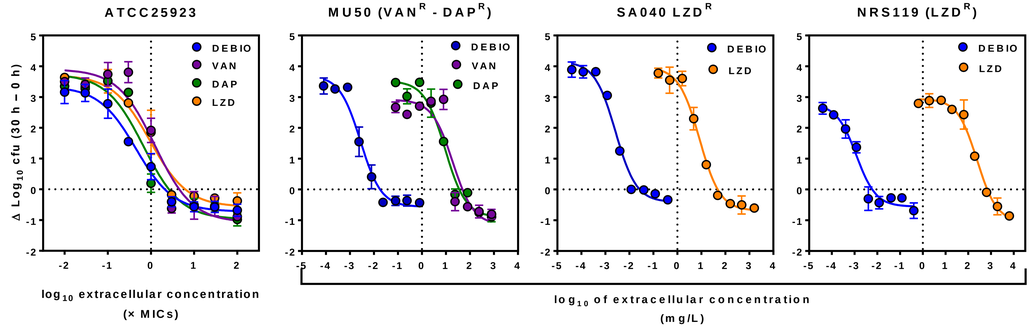
- C_s (relative potency): extracellular concentration resulting in no apparent bacterial growth (no. CFU at 30h = post-phagocytosis inoculum).
- E_{max} (maximal relative efficacy): CFU change (in log_{10} units) at 30 h from the post-phagocytosis inoculum as extrapolated for an infinitely large antibiotic concentration based on the Hill equation (slope factor set to 1)

Results

Strains, MICs, resistance patterns and intracellular pharmacodynamic parameters

Strain	Antibiotic	MIC (mg/L)	EUCAST categor.	C_s (95% CI)		Emax (95% CI)
				mg/L	xMIC	
ATCC25923	Debio 1452	0.004	nd	**0.02 (0.01 to 0.03)**	**4.94 (1.88 to 8.00)**	**-0.725 (-1.044 to -0.4054)**
	linezolid	2	S	13.48 (5.17 to 21.78)	6.74 (2.59 to 10.89)	-0.571 (-1.010 to -0.1305)
	vancomycin	1	S	4.65 (4.12 to 5.19)	4.65 (4.12 to 5.19)	-1.075 (-1.741 to -0.4101)
	daptomycin	1	S	2.65 (2.02 to 3.29)	2.65 (2.02 to 3.29)	-0.975 (-1.464 to -0.4857)
MU50	Debio 1452	0.004	nd	**0.02 (0.01 to 0.03)**	**5.30 (1.91 to 8.68)**	**-0.563 (-0.8670 to -0.2596)**
	vancomycin	8	R	51.97 (26.81 to 77.13)	6.50 (3.335 to 9.64)	-1.145 (-1.955 to -0.3351)
	daptomycin	8	R	37.11 (23.61 to 50.60)	4.64 (2.95 to 6.33)	-0.904 (-1.283 to -0.5259)
SA040 LZD^R	Debio 1452	0.004	nd	**0.03 (0.02 to 0.03)**	**6.86 (5.81 to 7.92)**	**-0.409 (-0.6414 to -0.1775)**
	linezolid	16	R	50.50 (29.93 to 71.08)	3.16 (1.87 to 4.44)	-0.692 (-0.9803 to -0.4046)
NRS119	Debio 1452	0.004	nd	**0.01 (0.00 to 0.01)**	**2.06 (0.54 to 3.58)**	**-0.556 (-0.8047 to -0.3080)**
	linezolid	64	R	684.49 (653.76 to 715.23)	10.7 (10.21 to 11.18)	-1.056 (-1.425 to -0.6865)

Concentration-responses by strain and antibiotic



- **Very high potency of Debio 1452 with MICs of 0.004 mg/L and C_s between 0.01 and 0.03 mg/L in broth or intracellularly against all the strains used in this study (C_s average: about 1,300 fold lower than the comparators for susceptible strains).**
- **An intracellular maximal efficacy (E_{max}) similar to that of other drugs tested (-0.4 and -0.7 log_{10} CFU decrease)**
- **No apparent effect of resistance mechanisms to other antibiotics in broth or intracellularly for the strains used.**

Discussion and Conclusions

- Debio 1452 is active against *S. aureus* phagocytized and thriving in human THP-1 monocytes, disregarding their resistant phenotypes to other currently used antistaphylococcal antibiotics.
- The intracellular relative potency (C_s) of Debio 1452 is close to its MIC in broth, which suggests a free penetration and an effective access to its bacterial target in phagocytes.
- The data suggest that Debio 1452 may constitute a useful alternative to most antistaphylococcal agents for acting against intracellular susceptible as well as multidrug resistant *S. aureus.*

References

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