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In vitro antimicrobial activity of AZD2563, a novel oxazolidinone, tested against *Staphylococcus aureus* and coagulase-negative staphylococci

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Revised abstract

Background: The escalating number of drug-resistant Gram-positive organisms world-wide urgently requires development of new molecular entities for infection chemotherapy. The oxazolidinones offer essentially complete spectra of activity against these strains, and a novel agent, AZD2563, has been recently described.
Methods: This study reports the testing of 384 *Staphylococcus aureus* (176 oxacillin-resistant [ORSA]) and 219 coagulase-negative staphylococci (CoNS) (of which 162 were oxacillin-resistant [OR-CoNS]) against AZD2563. Reference NCCLS microdilution and agar dilution tests were used and 10 comparator drugs included linezolid (LZD), quinupristin-dalfopristin (Q-D), vancomycin (VAN), three macrolides, clindamycin (CLI), gentamicin (GEN), oxacillin (OXAC), and levofloxacin (LEVX).
Results: Against *S. aureus* the following results were noted (MIC_{50}/MIC_{90}/% susceptible [≤ 4 µg/mL for AZD2563]): AZD2563 (1/2/100%), LZD (2/2/100%), Q-D (0.25/0.5/99%) and VAN (1/1/100%). Only 48–63% of the strains tested were susceptible to other comparator drugs such as the macrolides, CLI, quinolones and aminoglycosides. CoNS results were: AZD2563 (0.5/1/100%), LZD (1/1/100%), Q-D (0.25/0.5/100%), and VAN (1/2/100%). The AZD2563 MIC mode was two-fold lower than LZD for all 603 strains. ORSA and OR-CoNS strains were equally susceptible to both oxazolidinones as compared to oxacillin-susceptible strains. Reference agar and broth dilution AZD2563 MIC results were the same (100% ± one $\log_2$ dilution). Killing curve kinetic studies demonstrated bacteriostatic, concentration independent action of AZD2563 and indifference with VAN or GEN in combinations.
Conclusion: AZD2563 demonstrated antimicrobial activity with a potency slightly superior to LZD. Further investigation of *in vivo* and *in vitro* activity against other Gram-positive organisms is warranted.

Introduction

- Emerging and increasing resistance to antimicrobial agents among Gram-positive pathogens has been a prevailing challenge for antimicrobial chemotherapy for over a decade.
- Of particular concern are the sustained high rates of resistance to β-lactams and macrolides in *Streptococcus pneumoniae* and viridans group streptococci, the escalating resistance of enterococci to glycopeptides, and resistance to antimicrobials in staphylococci through various mechanisms (e.g. methicillin-resistance, VAN-intermediate *S. aureus* [VISA]).
- Various approaches have been investigated to address these issues, including streptogramin combinations, everninomicins, novel cephems, new quinolones, glyco- or lipo-peptides, and oxazolidinones.
- The objective of this investigation was to characterize the *in vitro* activity of AZD2563, a new oxazolidinone, against recent strains of staphylococci that include various resistance phenotypes and genotypes.

Methods

- Bacterial strains were derived from clinical isolates collected by the JMI Laboratories within the last 12 months, and consisted of 384 *S. aureus* (including 176 OXAC-resistant isolates [ORSA], and 10 VISA isolates) and 219 CoNS (of which 162 were resistant to OXAC). Each strain was identified independently by two laboratories.
- All strains were tested for susceptibility to antimicrobial agents using the broth microdilution protocol of the NCCLS (2000). Frozen-form broth microdilutions were obtained from TREK Diagnostics (Westlake, OH), and stored at -80°C until used.
- AZD2563 was obtained from AstraZeneca (Macclesfield, UK), and other antimicrobials were provided by their respective US manufacturers. Comparator agents were: LZD, Q-D, VAN, azithromycin (AZI), clarithromycin (CLA), erythromycin (ERY), CLI, LEVX and OXAC. NCCLS agar (Mueller–Hinton agar) MIC tests were also performed for comparative purposes.

Results

Table 1. *In vitro* activity of AZD2563 compared with that of 10 other antimicrobial agents tested against 384 strains of *S. aureus*.

Anti-microbial agent	OXAC-susceptible (n = 208)				OXAC-resistant (n = 176)				All strains (n = 384)			
	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	Range (µg/mL)	% susc.	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	Range (µg/mL)	% susc.	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	Range (µg/mL)	% susc.
AZD2563	1	2	0.25–4	N/D	1	2	0.25–2	N/D	1	2	0.25–4	N/D*
LZD	2	2	0.5–4	100.0	2	2	0.5–4	100.0	2	2	0.5–4	100.0
Q-D	0.25	0.5	0.06–1	100.0	0.5	1	0.25– > 8	97.7	0.25	0.5	0.06– > 8	99.0
VAN	1	1	0.25–1	100.0	1	1	0.5–2	100.0	1	1	0.25–2	100.0
AZI	≤ 2	> 8	≤ 2– > 4	81.7	> 4	> 4	≤ 2– > 4	8.5	> 4	> 4	≤ 2 – > 4	48.2
CLA	≤ 2	> 4	≤ 2– > 4	80.3	> 4	> 4	≤ 2– > 4	10.8	> 4	> 4	≤ 2– > 4	48.4
ERY	0.25	> 16	≤ 0.12–> 16	81.3	> 16	> 16	≤ 0.25–> 16	9.1	16	> 16	≤ 0.12–> 16	48.2
CLI	≤ 0.5	≤ 0.5	≤ 0.5–> 4	95.2	> 4	> 4	≤ 0.5–> 4	25.6	≤ 0.5	> 4	≤ 0.5–> 4	63.3
GEN	≤ 0.25	1	≤ 0.25–> 8	94.7	> 8	> 8	≤ 0.25–> 8	35.2	0.5	> 8	≤ 0.25–> 8	67.4
LEVX	0.12	0.5	0.03–> 8	94.7	8	> 8	0.06– > 8	9.7	0.25	> 8	0.03– > 8	55.7
OXAC	0.25	0.5	0.06–2	100.0	> 16	> 16	4– > 16	0.0	0.5	> 16	0.06–> 16	54.2

*N/D No breakpoint concentration has been defined for this new compound.

- AZD2563 was generally as active, or two-fold more active than LZD (MIC_{50} values 1 and 2 µg/mL for AZD2563 and LZD, respectively) against *S. aureus* (384 strains; Table 1).
- Although VAN and Q-D were more active weight-for-weight than both oxazolidinones, approximately 1% of strains were resistant to Q-D. All other agents showed activity against ≤ 68% of strains.
- Oxazolidinone, glycopeptide, and streptogramin potencies were independent of β-lactam (OXAC), macrolide, aminoglycoside, CLI, glycopeptide (VISA), and fluoroquinolone resistance.

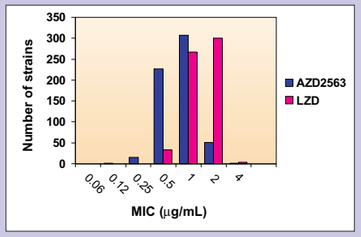
Table 2. *In vitro* activity of AZD2563 compared with that of 10 other antimicrobial agents tested against 219 strains of coagulase-negative staphylococci.

Anti-microbial agent	OXAC-susceptible (n = 57)				OXAC-resistant (n = 162)				All strains (n = 219)			
	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	Range (µg/mL)	% susc.	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	Range (µg/mL)	% susc.	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	Range (µg/mL)	% susc.
AZD2563	0.5	1	0.25–2	N/D	0.5	1	0.12–1	N/D	0.5	1	0.12–2	N/D*
LZD	1	1	0.5–2	100.0	1	2	0.5–2	100.0	1	1	0.5–2	100.0
Q-D	0.12	0.25	0.06-0.5	100.0	0.25	0.5	0.06–1	100.0	0.25	0.5	0.06–1	100.0
VAN	1	2	0.5–2	100.0	1	2	0.25–4	100.0	1	2	0.25–4	100.0
AZI	≤ 2	> 4	≤ 2– > 4	59.6	> 4	> 4	≤ 2– > 4	21.6	> 4	> 4	≤ 2 – > 4	31.5
CLA	≤ 2	> 4	≤ 2– > 4	61.4	> 4	> 4	≤ 2– > 4	21.6	> 4	> 4	≤ 2– > 4	32
ERY	0.25	> 16	≤ 0.12–>16	61.4	> 16	> 16	≤ 0.12–>16	21.6	> 16	> 16	≤ 0.12–>16	32
CLI	≤ 0.5	> 4	≤ 0.5–> 4	87.7	≤ 0.5	> 4	≤ 0.5–> 4	64.8	≤ 0.5	> 4	≤ 0.5–> 4	70.8
GEN	≤ 0.25	> 8	≤ 0.25–> 8	89.5	8	> 8	≤ 0.25–> 8	48.1	≤ 0.25	> 8	≤ 0.25–> 8	58.9
LEVX	0.12	8	0.06–> 8	84.2	2	> 8	0.06– > 8	50.6	0.5	> 8	0.06– > 8	59.4
OXAC	0.12	0.25	0.03–0.25	100.0	> 16	> 16	0.5– > 16	0.0	4	> 16	0.03–> 16	26.0

*N/D No breakpoint concentration has been defined for this new compound.

- All CoNS strains (n = 219) were susceptible to oxazolidinones, VAN and Q-D, but resistance was 28–74% for other agents tested (Table 2).
- CoNS strains were approximately two-fold more susceptible to AZD2563 than LZD (MIC_{50} values 0.5 and 1 µg/mL respectively).
- VAN and Q-D had similar spectra of activity against coagulase-negative staphylococci.
- Killing curve kinetic studies demonstrated bacteriostatic, concentration-independent action of AZD2563 and indifference with VAN and GEN combinations (data not shown).

Fig 1. MIC distributions for AZD2563 and LZD tested against 603 strains of staphylococci.



- Fig 1 shows the distribution of oxazolidinone MICs for the entire 603 staphylococcal isolates. AZD2563 MICs were consistently lower than those of LZD.

Table 3. Comparisons of broth microdilution MICs to those of the agar dilution method (NCCLS)* for two oxazolidinones tested against 120 isolates of Gram-positive cocci.

Antimicrobial/organisms (no. tested)	Broth/agar dilution MIC ratio				
	≤ 0.25	0.5	1	2	≥ 4
AZD2563					
S. pneumoniae (30)	0	0	30	0	0
Other streptococci (30)	0	13	17	0	0
Staphylococci (30)	0	0	30	0	0
Enterococci (30)	0	0	30	0	0
Total (120)‡	0	13	107	0	0
LZD					
S. pneumoniae (30)	0	0	29	1	0
Other streptococci (30)	0	15	12	3	0
Staphylococci (30)	0	0	30	0	0
Enterococci (30)	0	0	30	0	0
Total (120)‡	0	15	101	4	0

*Testing was by reference NCCLS methods.
†All of the AZD2563 results were within the ± one $\log_2$ dilution range and 89.2% were at a ratio of 1.
‡All of the control LZD results were within the ± one $\log_2$ dilution range and 84.2% were at a ratio of 1.

- To check for reproducibility of results across experimental protocols, microdilution MIC results were compared with results from agar dilution protocols. Both AZD2563 and LZD MICs were highly reproducible across these two methods (Table 3).

Conclusions

- AZD2563 is a new oxazolidinone with activity against all staphylococci tested in this study (MIC_{90} values 1–2 µg/mL).
- AZD2563 potency was equal or two-fold higher than LZD as determined by MIC_{50} and MIC_{90}.
- AZD2563 is a promising novel agent with activity against Gram-positive cocci including those resistant to other classes of antimicrobial agents.

Selected references

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