**In vitro** activity of a novel oxazolidinone, AZD2563, against randomly selected and multiresistant Gram-positive cocci

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The *in vitro* activity of AZD2563, a novel oxazolidinone, was assessed against 595 Gram-positive cocci, comprising recent surveillance isolates and a collection of resistant (including multiresistant), epidemiologically diverse isolates. The MICs of AZD2563 for staphylococci, pneumococci and enterococci had narrow ranges, 0.25–2 mg/L, with modal MICs of 1 mg/L for staphylococci and pneumococci, and 1–2 mg/L for enterococci. AZD2563 was equally active against the surveillance isolates and those that had been selected for their multiresistance to other agents. The MICs of AZD2563 were either the same as those of linezolid or two-fold lower.

**Introduction**

The increasing prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA), β-lactam- and macrolide-resistant pneumococci, and glycopeptide-resistant enterococci, is causing mounting concern. In response, the pharmaceutical industry continues to seek and develop new antibacterial agents. Amongst the most interesting of these are the oxazolidinones, which are synthetic antimicrobials with anti-Gram-positive activity. One oxazolidinone, linezolid, has recently entered use and represents the first member of a genuinely new class of antimicrobials to be launched in 30 years. Linezolid is active at 1–4 mg/L against virtually all Gram-positive cocci, with the exception of a few mutants selected during therapy. It requires bd dosing.

We report here on the activity against staphylococci, pneumococci and enterococci, of a novel long half-life oxazolidinone, AZD2563. The bacteria tested comprised both randomly selected isolates obtained during surveillance, and isolates selected for resistance to first-line agents. The latter group were also selected for epidemiological diversity, were from a wide geographical spread of hospitals and included a variety of phage types of *S. aureus* and serotypes of *Streptococcus pneumoniae*.

**Materials and methods**

**Bacteria**

The activity of AZD2563 was assessed against 595 Gram-positive isolates comprising two groups, namely (i) randomly selected isolates collected during a resistance prevalence survey in the UK, and (ii) resistant (including multiresistant) isolates selected from among those referred to the Antibiotic Resistance Monitoring and Reference Laboratory (ARMRL) for confirmation of resistance. The resistant isolates were selected as having come from geographically diverse UK hospitals, and isolates of *S. aureus* and *S. pneumoniae* were further selected so as to include a range of phage types and serogroups/types, respectively. The 595 isolates comprised 184 isolates of *S. aureus* (90 surveillance, 94 selected), 60 isolates of coagulase-negative staphylococci (CNS) (from the prevalence survey only), 176 isolates of *S. pneumoniae* (82 surveillance, 94 selected) and 175 isolates of *Enterococcus* spp. (81 surveillance, 94 selected).

**Antibiotics**

AZD2563 (Figure 1) was from AstraZeneca (Macclesfield, UK); linezolid was from Pharmacia (Milton Keynes, UK);
levofloxacin and quinupristin/dalfopristin were from Aventis (West Malling, UK); oxacillin, penicillin, erythromycin, gentamicin and vancomycin were from Sigma (Poole, UK).

Susceptibility tests

MICs were determined by the agar dilution method on Mueller–Hinton agar (Oxoid, Basingstoke, UK) with inocula of $10^4$–$10^5$ cfu/spot. For tests with β-lactams against staphylococci, the medium was supplemented with 2% NaCl, while the medium for streptococci was supplemented with 5% whole sheep blood (TCS Microbiology, Buckingham, UK). All cultures were incubated in air with the exception of pneumococci, which were incubated in an atmosphere enriched with 5% CO2. All results were read after incubation at $35^\circ C$ for 24 h. Except for the use of solid media and incubation in an atmosphere containing 5% CO2 with pneumococci, these methods corresponded to those of the NCCLS, whose breakpoints were used.6,7

Results

Staphylococcus aureus

The 90 surveillance isolates of S. aureus were from 24 hospitals. Approximately 38% were resistant to oxacillin at the NCCLS breakpoint concentration of 2 mg/L (Figure 2a), which approximately matches the MRSA prevalence rate for bacteraemic isolates in England and Wales.8,9 Most of the surveillance isolates of MRSA were resistant to erythromycin and levofloxacin, probably reflecting the predominance of the epidemic MRSA (EMRSA) strains EMRSA-15 and -16, which are usually resistant to these drugs, and which account for over 95% of the MRSA bacteraemias in England and Wales.10 Only 3.3% of the surveillance isolates of S. aureus were resistant to gentamicin (Figure 2a).

The 94 selected S. aureus were from 71 hospitals. They comprised 58 MRSA and 36 methicillin-susceptible S. aureus (MSSA). The MSSA comprised 19 phage types, whereas the MRSA exhibited 25 phage reaction patterns, many of which were, however, variants of those seen with EMRSA-15 and -16. Resistance rates to erythromycin, levofloxacin and gentamicin were 91%, 79% and 12%, respectively, among the MRSA, and 39%, 14% and 8% among the MSSA. The overall rates of resistance of the selected isolates to these agents are shown in Figure 2(a).

The MICs of AZD2563 for all the isolates of S. aureus tested were between 0.5 and 2 mg/L, with modal values of 1 mg/L for both the surveillance and selected isolates (Figure 3a). MICs of linezolid were in the range 0.5–4 mg/L, with modal values of 2 mg/L for both groups (Figure 3b). The activity of AZD2563 against MRSA was equivalent to that against MSSA, again with modal MICs of 1 mg/L for both groups, with actual MICs of 1 mg/L recorded for 79% of the MRSA and 75% of the MSSA. Similarly, the modal MIC of linezolid was 2 mg/L for both MRSA and MSSA. For 50 of
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The 184 *S. aureus* isolates, the MICs of AZD2563 and linezolid were equal, whereas for 131 isolates, the MIC of AZD2563 was two-, or rarely, four-fold below that of linezolid. Only three isolates were more susceptible to linezolid than AZD2563 (Figure 4a).

Coagulase-negative staphylococci

The 60 surveillance isolates of CNS were from 22 hospitals. They showed a greater degree of antimicrobial resistance than the surveillance isolates of *S. aureus*, with 72% resistant to oxacillin at the NCCLS breakpoint concentration of 0.25 mg/L. Resistance rates to erythromycin, levofloxacin, gentamicin and quinupristin/dalfopristin, were 50%, 32%, 37% and 3%, respectively (Figure 2a).

The MICs of AZD2563 for these CNS were in the range 0.5–2 mg/L, with a mode of 1 mg/L (Figure 3a); those of linezolid were in the range 1–2 mg/L with a mode of 1 mg/L (Figure 3b). AZD2563 and linezolid were equally active against oxacillin-susceptible and -resistant CNS, with a modal MIC of 1 mg/L for each group (data not shown). For 50 of the 60 CNS, the MICs of AZD2563 and linezolid were equal, whereas the MIC of AZD2563 was a two-fold dilution below that of linezolid for 10 isolates (Figure 4b).

**Streptococcus pneumoniae**

The 82 surveillance isolates of *S. pneumoniae* were from 24 hospitals. Intermediate resistance to penicillin (MIC 0.125–1 mg/L) was seen in 7% of the isolates, with none being fully penicillin resistant. Resistance to erythromycin was seen in 14% of the isolates while resistance to levofloxacin was not seen (Figure 2b). The 94 selected isolates were from 63 hospitals and comprised 20 serogroups/types. Reduced susceptibility to penicillin was seen in 78% (53% intermediate, 25% resistant), whereas 37% were resistant to erythromycin (Figure 2b). Levofloxacin resistance was seen in six isolates, all of which required MICs of ≥8 mg/L. The MICs of AZD2563 for *S. pneumoniae* ranged from 0.25 to 2 mg/L, with a mode of 1 mg/L (Figure 3a); those of linezolid were in the range 0.5–2 mg/L with a mode of 1 mg/L (Figure 3b). The activities of AZD2563 and linezolid were unaffected by resistance to other agents, their modal MICs remaining at 1 mg/L for penicillin-susceptible, -intermediate and -resistant isolates, and for those that were either susceptible or resistant to erythromycin and levofloxacin (data not shown). For 90 of the 176 isolates, the MICs of AZD2563 and linezolid were equal, whereas the MICs of AZD2563 were two-fold lower than those of linezolid for 80 isolates. Only six isolates were more susceptible to linezolid than AZD2563 (Figure 4c).

**Figure 3.** MICs of AZD2563 (a) and linezolid (b). Staphylococci: surveillance isolates of *S. aureus* (white bars), selected isolates of *S. aureus* (black bars) and surveillance isolates of CNS (striped bars). Pneumococci: surveillance isolates of *S. pneumoniae* (white bars) and selected isolates of *S. pneumoniae* (black bars). Enterococci: surveillance isolates of *E. faecalis* (white bars), selected isolates of *E. faecalis* (black bars) and selected isolates of *E. faecium* (striped bars).
Enterococcus spp.

The 81 surveillance isolates of enterococci were from 23 hospitals and comprised 75 Enterococcus faecalis and six Enterococcus faecium. Among the E. faecalis isolates, 77% were erythromycin resistant and 17% had high-level gentamicin resistance. One isolate of E. faecium was resistant to vancomycin, but all other surveillance isolates of both E. faecium and E. faecalis were susceptible (Figure 2c). The 94 selected enterococcal isolates were from 48 hospitals and comprised 41 E. faecalis, 45 E. faecium and eight Enterococcus gallinarum. Among the E. faecalis isolates, 90% were erythromycin resistant, 59% had high-level gentamicin resistance and 63% were resistant to vancomycin. Among the E. faecium isolates, 98% were erythromycin resistant, 40% had high-level gentamicin resistance and 82% were resistant to vancomycin (Figure 2c).

The MICs of AZD2563 were in the range 1–2 mg/L for enterococci, with a mode of 1 mg/L for the selected isolates of E. faecalis, and a mode of 2 mg/L for the surveillance isolates of E. faecalis and selected isolates of E. faecium (Figure 3a). MICs of AZD2563 for the eight selected isolates of E. gallinarum were either 1 mg/L (two isolates) or 2 mg/L (six isolates). MICs of linezolid were in the range 1–4 mg/L, with a mode of 2 mg/L for all groups of enterococci (Figure 3b). Resistance to other agents did not affect the activity of AZD2563 or linezolid, which both retained equal activity against vancomycin-susceptible and -resistant enterococci. For 134 of the 175 isolates, the MICs of AZD2563 and linezolid were equal, whereas the MICs of AZD2563 were two-fold lower than those of linezolid for the remaining 41 isolates (Figure 4d).

Discussion

The novel oxazolidinone AZD2563 has good activity against staphylococci, pneumococci and enterococci, with MICs generally either the same as, or two-fold lower than those of linezolid (Figure 4). These conclusions applied to both surveillance isolates and isolates selected to represent multi-resistant strains. As with linezolid, AZD2563 retained full activity against methicillin-resistant staphylococci, penicillin-and/or erythromycin-resistant pneumococci, and vancomycin-resistant enterococci. This was not unexpected, since oxazolidinones inhibit protein synthesis at a unique stage, by inhibiting formation of the initiation complex. Although oxazolidinones presently exhibit near-comprehensive anti-Gram-positive activity, there have been a few reports of resistance emerging to linezolid during therapy of enterococcal infections, and a single report of resistance in a strain of MRSA. It is thus essential that the susceptibility of Gram-positive pathogens to oxazolidinones is monitored prospectively. All of six linezolid-resistant enterococci (all from patients treated with linezolid) so far referred to the ARMRL were cross-resistant to AZD2563 (A. P. Johnson, M. Warner & D. M. Livermore, unpublished results). The early detection and reporting of such cases, together with analysis of relevant clinical information, may allow risk factors for the emergence of oxazolidinone resistance to be identified. Early experience with linezolid indicates that these include under-dosage, sequestered sites, indwelling devices and protracted therapy. Confirmation of these findings may allow guidelines for treatment with oxazolidinones to be optimized, with the aim not only of obtaining maximal therapeutic benefit, but also of minimizing the emergence of resistance, thus prolonging the clinical usefulness of this new class of antimicrobial agents.

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