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Review

Linezolid update: Stable *in vitro* activity following more than a decade of clinical use and summary of associated resistance mechanisms

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ABSTRACT

Linezolid, approved for clinical use since 2000, has become an important addition to the anti-Gram-positive infection armamentarium. This oxazolidinone drug has *in vitro* and *in vivo* activity against essentially all Gram-positive organisms, including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE). The *in vitro* activity of linezolid was well documented prior to its clinical application, and several ongoing surveillance studies demonstrated consistent and potent results during the subsequent years of clinical use. Emergence of resistance has been limited and associated with invasive procedures, deep organ involvement, presence of foreign material and mainly prolonged therapy. Non-susceptible organisms usually demonstrate alterations in the 23S rRNA target, which remain the main resistance mechanism observed in enterococci; although a few reports have described the detection of *cfr*-mediated resistance in *Enterococcus faecalis*. *S. aureus* isolates non-susceptible to linezolid remain rare in large surveillance studies. Most isolates harbour 23S rRNA mutations; however, *cfr*-carrying MRSA isolates have been observed in the United States and elsewhere. It is still uncertain whether the occurrences of such isolates are becoming more prevalent. Coagulase-negative isolates (CoNS) resistant to linezolid were uncommon following clinical approval. Surveillance data have indicated that CoNS isolates, mainly *Staphylococcus epidermidis*, currently account for the majority of Gram-positive organisms displaying elevated MIC results to linezolid. In addition, these isolates frequently demonstrate complex and numerous resistance mechanisms, such as alterations in the ribosomal proteins L3 and/or L4 and/or presence of *cfr* and/or modifications in 23S rRNA. The knowledge acquired during the past decades on this initially used oxazolidinone has been utilized for developing new candidate agents, such as tedizolid and radezolid, and as linezolid patents soon begin to expire, generic brands will certainly become available. These events will likely establish a new chapter for this successful class of antimicrobial agents.

Contents

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1. Introduction

During the last decades, only a few new antimicrobial agents have been approved for clinical use by the United States (USA) Food and Drug Administration (FDA), European Medicines Agency (EMA) and other regulatory agencies (Gould and Bal, 2013). Among those agents with Gram-positive organism coverage, ceftaroline and telavancin are improved analogues of known scaffolds, while daptomycin and linezolid are its sole representative molecules of entirely new classes of antimicrobials (*i.e.* lipopeptides and oxazolidinones, respectively). Linezolid received a priority review by the FDA, which approved this drug for clinical use in March of 2000, and linezolid has become an important addition to the anti-Gram-positive infection armamentarium. Indications consist of treatment for uncomplicated and complicated skin and skin structure infections (cSSSI) and hospital- and community-acquired pneumonia caused by Gram-positive pathogens (Zyvox, 2010). Linezolid is also indicated for the treatment of vancomycin-resistant *Enterococcus faecium* (VRE) infections (including cases with concurrent bacteremia).

Since the ribosome represents the core for protein synthesis in all living cells, numerous antimicrobial agents targeting the peptidyltransferase centre (PTC) of the large ribosomal subunit have been developed. Targeting the PTC remains one of the main advantages of the oxazolidinones due to the number of rRNA genes, which minimizes the emergence of resistance (Toh et al., 2007). Oxazolidinones were initially known to affect protein synthesis during the initiation phase of translation (Lin et al., 1997; Shinabarger et al., 1997). A later study offered additional insights and demonstrated that linezolid interacts with the 23S rRNA (A2602; *Escherichia coli* numbering used throughout) and prevents binding or proper placement of aminoacyl-tRNA in the PTC site (Leach et al., 2007). However, linezolid also seems to interact with ribosomal protein L27, ribosomal-associated protein LepA and tRNA (Colca et al., 2003). These interactions are still unknown, but could be associated with ribosome formation and fidelity of translation (Colca et al., 2003).

Linezolid is available in intravenous and oral formulations, which have provided this agent as an attractive alternative for treating numerous infection types, including respiratory tract infection caused by methicillin-resistant *Staphylococcus aureus* (MRSA) and other serious multidrug-resistant (MDR) infections due to vancomycin-resistant enterococci (VRE). The clinical and commercial success of linezolid has prompted many pharmaceutical companies to investigate and develop oxazolidinone-like compounds (Michalska et al., 2013). Several molecules have been developed as candidates, but only tedizolid and radezolid have advanced into clinical trials (Shaw and Barbachyn, 2011). This review provides a summary update for linezolid with regard to the pre- and post-FDA approval study results related to *in vitro* antimicrobial activity and spectrum, and development and dissemination of resistance mechanisms. A thorough review on the activity *in vitro* of other oxazolidinones tested against Gram-positive isolates, including linezolid-resistant strains, was recently published and this topic will not be addressed this topic (Shaw and Barbachyn, 2011).

2. Antimicrobial spectrum and activity

2.1. Pre-FDA approval

Investigational studies performed during the development of linezolid documented a broad and potent antimicrobial activity against Gram-positive organisms. Linezolid displayed *in vitro* inhibitory activities against numerous clinically relevant bacterial species, including staphylococci (methicillin-susceptible and -resistant), enterococci (vancomycin-susceptible and -resistant), streptococci, *Corynebacterium* spp., *Mycobacterium tuberculosis* and some species of anaerobic bacteria (Zurenko et al., 1996).

Table 1 summarizes the MIC_{50} and MIC_{90} values for linezolid when tested against Gram-positive clinical isolates obtained from studies performed prior to the FDA approval. Overall, these studies reported linezolid MIC results between 0.25 and 4 mg/L, and non-susceptible results were only initially obtained against laboratory-derived mutants after numerous daily passaging experiments in drug containing media. During the pre-FDA approval era, linezolid demonstrated a normal MIC distribution (*i.e.* Gauss curve) when tested against clinical Gram-positive pathogens, which was confirmed by several local and multicentre *in vitro* studies conducted by investigators on several continents (Zurenko et al., 1996; Jones et al., 1996; Wise et al., 1998; von Eiff and Peters, 1999; Noskin et al., 1999; Johnson et al., 2000; Rybak et al., 1998, 2000).

Overall, studies evaluating the *in vitro* activity and spectrum of linezolid have reported MIC_{50} values of 2–4, 2–4 and 1–2 mg/L when tested against staphylococci, enterococci and streptococci, respectively. However, Wise et al. (1998) published linezolid MIC results when tested against staphylococci and enterococci lower than other authors, with highest linezolid values at 1 mg/L (see Table 1). In addition, Jones et al. (1996) published linezolid MIC_{50} values (1 mg/L) against enterococci similar to those reported by Wise et al. (1998), which were, in general, lower than those from other publications.

The variations observed in the linezolid MIC results in these studies can, perhaps, be explained by the different guidelines (EUCAST *versus* CLSI) and methods (broth microdilution and agar dilution *versus* Etest). Also, some studies utilized agar dilution techniques and IsoSensititre media (Wise et al., 1998; von Eiff and Peters, 1999; Johnson et al., 2000), while other investigators made use of broth microdilution methods and the Mueller-Hinton medium (Zurenko et al., 1996; Jones et al., 1996; Noskin et al., 1999; Rybak et al., 1998, 2000). Regardless, the different guidelines, methods and media utilized only indicate the presence of several uncontrolled variables among early studies and imply methodological differences, and there was no correlation of the method applied with the MIC results obtained. The MIC endpoint reading itself can be another potential element causing variability. Trailing is a common phenomenon when testing linezolid, requiring additional expertise for determining the correct endpoint value as currently stated in some international standardized methods (Biedenbach and Jones, 1997, 2001, 2003; Poppe et al., 2006; Worth et al., 1996; Tenover et al., 2007).

These technical difficulties along with the lack of reading standards when testing such drug for susceptibility provide additional challenges for clinical microbiologists and research personnel. During the January 2006 Clinical and Laboratory Standards Institute (CLSI) meeting (Miami, FL), the Staphylococci Working Group proposed additional language in the M02, M07 and M100 documents to provide information detail on how to read MIC results and zones of inhibition in the presence of so-called "trailing endpoints" (Clinical and Laboratory Standards Institute, 2006). These changes were proposed to contain the following recommendation: "For some antimicrobial agents (such as for chloramphenicol, clindamycin, erythromycin, linezolid and tetracycline), trailing growth can make endpoint determination difficult. In such cases, the MIC should be read at the first well that shows a prominent reduction in growth. Tiny buttons of growth should be ignored". However, these proposed languages have not been incorporated into the M02, M07 or M100 documents by the time this review article was written. According to the summary minutes of the 2012 CLSI meeting, this recommendation will be published in the M07-10 in 2015 (Clinical and Laboratory Standards Institute, 2012a, 2014).