Diagnostic Microbiology and Infectious Disease xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Determination of Tedizolid susceptibility interpretive criteria for gram-positive pathogens according to clinical and laboratory standards institute guidelines

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ARTICLE INFO

Article history:
Received 9 February 2017
Received in revised form 26 October 2017
Accepted 29 October 2017
Available online xxxx

Keywords:
Antibacterial
Breakpoints
Gram-positive
Susceptibility criteria
Tedizolid

ABSTRACT

For effective antibacterial therapy, physicians require qualitative test results using susceptibility breakpoints provided by clinical microbiology laboratories. This article summarizes the key components used to establish the Clinical Laboratory Standards Institute (CLSI) breakpoints for tedizolid. First, *in vitro* studies using recent surveillance and clinical trial isolates ascertained minimal inhibitory concentration (MIC) distributions against pertinent organisms, including staphylococci, streptococci, and enterococci. Studies in animal models of infection determined rates of antibacterial efficacy and survival following administration of tedizolid phosphate at doses equivalent to those in humans. Pharmacokinetic and pharmacodynamic analyses examined the relationship between plasma concentrations and MICs against the target organism. Finally, clinical trials assessed clinical and microbiologic outcomes by MIC. All these data were evaluated and combined to obtain the ratified CLSI susceptibility criteria for tedizolid of ≤0.5 μg/mL for *Staphylococcus aureus*, *Streptococcus pyogenes, Streptococcus agalactiae,* and *Enterococcus faecalis* and ≤0.25 μg/mL for *Streptococcus anginosus* group.

1. Introduction

Tedizolid phosphate is an oxazolidinone prodrug that is rapidly converted *in vivo* by phosphatases to the active antibacterial tedizolid (Flanagan et al., 2014b; Ong et al., 2014). Like other oxazolidinones, the antibacterial activity of tedizolid arises from inhibition of protein synthesis resulting from binding to the 23S rRNA of the 50S ribosomal subunit (Locke et al., 2014; Zhanel et al., 2015). Based on Phase 3 trial results, which demonstrated that tedizolid (200 mg once a day [qd] for 6 days) was noninferior to linezolid (600 mg twice a day [bid] for 10 days) and was generally well tolerated in patients with acute bacterial skin and skin structure infections (ABSSSI) (Moran et al., 2014; Prokocimer et al., 2013), tedizolid has been approved for the treatment of ABSSSI in adults (Sivextro, 2015, 2016).

Tedizolid has demonstrated potent activity against Gram-positive pathogens such as staphylococci (including methicillin-resistant *Staphylococcus aureus* [MRSA]), streptococci, and enterococci (including vancomycin-resistant enterococci [VRE]) and is generally at least 4-fold more potent than linezolid as determined by *in vitro* susceptibility tests (Brown and Traczewski, 2010; Sahm et al., 2015; Schaadt et al., 2009; Zurenko et al., 2014).

Mechanisms of resistance to oxazolidinones, such as linezolid, include chromosomal mutations affecting 23S rRNA (Prystowsky et al., 2001; Tsiodras et al., 2001), mutations in genes encoding ribosomal proteins L3 and L4 (Long and Vester, 2012), presence of the plasmid-borne ribosomal methyltransferase gene *cfr* (Kaminska et al., 2010; Long et al., 2006), and expression of the resistance gene *optrA* (Wang et al., 2015). However, comparative studies have demonstrated that tedizolid retains antibacterial activity against linezolid-resistant Gram-positive pathogens, including strains with the *cfr* gene and lacking chromosomal mutations (Locke et al., 2010, 2014; Shaw et al., 2008).

In the United States, tedizolid is indicated for the treatment of ABSSSI caused by susceptible isolates of *Staphylococcus aureus* (including MRSA and methicillin-susceptible *S. aureus* [MSSA] isolates), *Streptococcus pyogenes, Streptococcus agalactiae, Streptococcus anginosus* group, and *Enterococcus faecalis.* Susceptibility test results from the clinical laboratory can help determine the likelihood that tedizolid will be effective in treating infection.

As a guide to effective antimicrobial therapy, qualitative test results using susceptibility breakpoints (susceptible, susceptible-dose dependent, intermediate, nonsusceptible, or resistant) are provided to physicians by clinical microbiology laboratories. *In vitro* susceptibility test interpretive criteria are established by the US Food and Drug Administration and consensus organizations, such as the Clinical Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing, with periodic revision as new data become

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https://doi.org/10.1016/j.diagmicrobio.2017.10.023
0732-8893/

available (Clinical and Laboratory Standards Institute (CLSI), 2016; European Committee on Antimicrobial Susceptibility Testing, 2016). To establish breakpoints, CLSI guidance requires data to be provided in three categories: pharmacologic data (pharmacokinetic [PK] and pharmacodynamic [PD]) demonstrating the relationship between plasma concentrations and the minimal inhibitory concentration (MIC) against the target organism (preclinical and clinical data); microbiologic data, which include *in vitro* activity (MIC distributions) against recent isolates of pertinent organisms; and clinical and microbiologic data showing the correlation of outcomes by MIC from clinical trials (Clinical and Laboratory Standards Institute (CLSI), 2008, 2016).

Susceptibility breakpoints for tedizolid against Gram-positive pathogens have been established and approved by the CLSI as follows (Clinical and Laboratory Standards Institute (CLSI), 2008, 2016): *S. aureus,* ≤0.5 μg/mL (susceptible), 1 μg/mL (intermediate), and ≥2 μg/mL (resistant); *S. pyogenes, S. agalactiae,* and *E. faecalis,* ≤0.5 μg/mL (susceptible); and *S. anginosus* group, ≤0.25 μg/mL (susceptible). Mechanisms of resistance have thus far only been seen in staphylococci, are rare for enterococci spp., and have not been observed for streptococci spp. The breakpoints will be revised should mechanisms of resistance emerge in streptococci and enterococci spp.

Intermediate and resistant breakpoints are not available streptococci and enterococci spp. as article summarizes the key data provided to the CLSI subcommittee in support of the proposed, and now granted, tedizolid breakpoints. This background information should be of assistance to investigators, microbiologists, and clinicians in understanding the relevance of these breakpoints for clinical decision making.

2. Pharmacokinetic and pharmacodynamic data

2.1. Activity in murine models

In vivo experiments were conducted in a series of systemic and localized murine infection models to provide comparative data for the efficacy of tedizolid against a variety of Gram-positive pathogens. These animal data are critical to show the efficacy of tedizolid in different models using organisms with defined MICs and are considered a key part of the process of setting breakpoints.

2.1.1. Systemic infection – S. aureus and methicillin-resistant, coagulase-negative staphylococci (Merck & Co., Inc., Kenilworth, NJ, USA; data on file)

Four studies were performed to compare the efficacy of tedizolid and linezolid in animal models of systemic infection by staphylococci; MIC values for isolates used in these studies ranged from 0.125 to 0.5 μg/mL for tedizolid and 0.5 to 8.0 μg/mL for linezolid. Results of a mouse *S. aureus* infection study that included 3 strains of MSSA and 2 strains of MRSA demonstrated that the effective dose of oral tedizolid preventing lethality in 50% of animals (ED_{50}) was 2- to 4-fold lower than for linezolid (ED_{50}: 3.2–7.6 mg/kg *vs.* 9.6–21.4 mg/kg). With intravenous (IV) administration, tedizolid was 4–9-fold more potent than linezolid (ED_{50}: 1.5–4.3 mg/kg *vs.* 7.7–29.1 mg/kg). Similar results were reported in a methicillin-resistant coagulase-negative staphylococci systemic infection model and in a neutropenic model of systemic infection induced by VRE or vancomycin-susceptible enterococci. In a mouse septicemia model induced by a linezolid-resistant MRSA strain, CM/05, carrying the *cfr* resistance gene, oral treatment with tedizolid (20 mg/kg) resulted in 100% survival compared with a maximum of 80% survival with linezolid (50 mg/kg).

2.1.2. Systemic infection – S. pneumoniae (Choi et al., 2012)

Tedizolid phosphate and linezolid were tested against 4 penicillin-resistant *S. pneumoniae* strains in systemic infections in mice. Tedizolid phosphate was effective by oral and IV routes in preventing lethality against all streptococcal strains tested and was ~2-fold more potent than linezolid by the oral route and up to 8-fold more potent than linezolid by the IV route.

2.1.3. Localized infection – neutropenic thigh model (Louie et al., 2011)

In skin and soft tissue *S. aureus* infection in mice, tedizolid phosphate (10–80 mg/kg) was consistently more effective than linezolid at lower doses (10–150 mg/kg) after oral administration. The area under the free concentration-time curve (*f*AUC)/MIC ratio for tedizolid phosphate to achieve stasis in a neutropenic mouse thigh model of MRSA (ATCC 33591) infection was 49.3. In the same model, intraperitoneal treatment with tedizolid phosphate was highly active against MSSA and community-acquired MRSA (CA-MRSA) strains. The doses required to achieve stasis, 1 $\log_{10}$, or 2 $\log_{10}$ CFU reductions were similar for the 2 strains, and the resultant *f*AUC/MIC ratios to achieve stasis were 49.1 for MSSA and 47.1 for CA-MRSA. Whereas tedizolid phosphate doses of 37.6 and 66.9 mg/kg/day resulted in stasis and 1 $\log_{10}$ CFU/g decreases in bacterial densities, respectively, linezolid did not result in stasis at doses up to 150 mg/kg/day, which had previously been effective (Andes et al., 2002).

2.1.4. Localized infection – immunocompetent thigh model (Keel et al., 2012)

The efficacy profiles of tedizolid and linezolid were examined in an immunocompetent murine thigh model against 4 MRSA strains (3 hospital-associated MRSA [HA-MRSA], including 1 vancomycin-resistant [VRSA] and 1 CA-MRSA) and 1 MSSA strain. Treatments were intended to simulate a human steady state $fAUC_{0-24}$ of 200 mg once every 24 hours (q24h) for tedizolid phosphate or 600 mg q12h for linezolid over a 3-day treatment period. MIC values ranged from 0.25 to 0.5 μg/mL for tedizolid and 2 to 4 μg/mL for linezolid. PK determinations indicated that for tedizolid phosphate, the actual $fAUC_{0-24}$ was 2.99 μg·h/mL (lower than the targeted human $fAUC_{0-24}$ value of 5.2 μg·h/mL); for linezolid, the actual $fAUC_{0-24}$ was 144 μg·h/mL (higher than the targeted human $fAUC_{0-24}$ value of 96.6 μg·h/mL). Nevertheless, these regimens for tedizolid phosphate and for linezolid reduced the counts of all staphylococcal isolates. There were no statistical differences between tedizolid phosphate and linezolid over the 72-hour period.

2.1.5. Localized infection – neutropenic pneumonia model (Lepak et al., 2012)

The *in vivo* PK/PD characteristics of tedizolid and linezolid were characterized and compared in a neutropenic mouse pneumonia model against 11 isolates of *S. aureus*, including 1 HA-MRSA, 6 CA-MRSA, and 4 MSSA strains. The dose needed to achieve net stasis or 1 $\log_{10}$ kill was determined, and *f*AUC/MIC was calculated. When binding to plasma proteins was considered, the mean *f*AUC/MIC values for tedizolid and linezolid were similar at 20 and 19, respectively, and *f*AUC/MIC values associated with 1 $\log_{10}$ kill reduction were roughly 2-fold higher than those needed for stasis. The *f*AUC/MIC values associated with net stasis and 1 $\log_{10}$ kill were similar for all strains tested.

2.1.6. Localized infection – immunocompetent pneumonia model (Tessier et al., 2012)

The antibacterial efficacies of tedizolid, linezolid, and vancomycin regimens simulating human epithelial lining fluid exposures were compared against 1 HA-MRSA and 2 CA-MRSA strains in an immunocompetent mouse pneumonia model. MIC values were 0.5 μg/mL for tedizolid and 2 to 8 μg/mL and 0.5 to 1 μg/mL for linezolid and vancomycin, respectively. Simulated human exposures (20 mg/kg tedizolid phosphate q24h, 120 mg/kg linezolid q12h, or 25 mg/kg vancomycin q12h) resulted in similar antibacterial efficacy for tedizolid phosphate and linezolid, but both antibacterial efficacy and survival were poorer with vancomycin than with either oxazolidinone.

2.1.7. Infection models: summary

The results of these animal efficacy studies support the *in vivo* efficacy of tedizolid in reducing bacterial counts and mortality rates. Simulating human exposures to the standard therapeutic dose of 200 mg qd

Please cite this article as: Bensaci M, et al, Determination of Tedizolid susceptibility interpretive criteria for gram-positive pathogens according to clinical and laboratory s..., Diagn Microbiol Infect Dis (2017), https://doi.org/10.1016/j.diagmicrobio.2017.10.023