Diagnostic Microbiology and Infectious Disease 87 (2017) 338–342

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

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

Antimicrobial Susceptibility Studies

Time-kill determination of the bactericidal activity of telavancin and vancomycin against clinical methicillin-resistant *Staphylococcus aureus* isolates from cancer patients

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ARTICLE INFO

Article history:
Received 14 September 2016
Received in revised form 7 December 2016
Accepted 11 December 2016
Available online 16 December 2016

Keywords:
Telavancin
Vancomycin
MRSA
Cancer patients
Time-kill
Bactericidal activity

ABSTRACT

The bactericidal activity of vancomycin and telavancin was compared against 4 clinical methicillin-resistant *Staphylococcus aureus* isolates recently recovered from cancer patients, using minimum bactericidal concentration (MBC):MIC ratios and time-kill studies. All 4 isolates were susceptible to both agents based on individual MIC values. The 2 methodologies for assessing bactericidal activity produced variable results. Telavancin appeared to have somewhat better bactericidal activity than vancomycin based on narrower MBC:MIC ratios. However, based on the results of the time-kill studies, neither agent demonstrated reliable bactericidal activity (defined as a ≥3 $\log_{10}$ reduction of the starting inoculum at the end of 24 hours) against these organisms. These findings might be of some therapeutic importance in certain clinical settings and/or specific patient populations (such as febrile neutropenic patients) in whom potent bactericidal activity is either desired or preferred.

1. Introduction

Bacterial infections occur frequently in patients with cancer, more often in those with hematologic malignancies and profound neutropenia, but also in patients with solid tumors who have adequate neutrophil counts (Avritscher et al. 2014; Freifeld et al. 2011; Virizuela et al. 2016). Current data indicate that gram-positive organisms predominate in this setting, and account for ~60–70% of monomicrobial bacterial infections (Klastersky et al. 2007; Montassier et al. 2013; Nesher and Rolston 2014). The organisms isolated most often are the coagulase-negative staphylococci (CoNS), *S. aureus*, and viridans group streptococci, (Rolston et al. 2014a). Vancomycin has been the agent of choice for the treatment of most documented gram-positive infections in cancer patients and has also been recommended as empiric therapy when gram-positive infections are strongly suspected (Baden et al. 2012; Freifeld et al. 2011). At many institutions, more than 90% of CoNS are methicillin-resistant, more than 50% of *S. aureus* isolates are methicillin-resistant (MRSA), and many isolates also have reduced susceptibility (MIC ≥1.0 μg/mL) to vancomycin (Rolston et al. 2015). Tolerance to vancomycin (defined as a minimum bactericidal concentration [MBC] ≥32 times the MIC) also occurs occasionally (Safdar and Rolston 2006). Responses to vancomycin have been shown to be suboptimal when such organisms are encountered (Mahajan et al. 2012; Sakoulas et al. 2004). National guidelines for the management of infections caused by MRSA recommend the use of alternative agents instead of vancomycin when such isolates are encountered (Liu et al. 2011). In the past few years, several new agents with in vitro activity against MRSA have been developed including daptomycin, linezolid, ceftaroline, telavancin, dalbavancin, and oritavancin (Rodvold and McConeghy 2014). Most of these have not been clinically evaluated in cancer patients.

Our institution (The University of Texas MD Anderson Cancer Center) is a National Cancer Institute–designated Comprehensive Cancer Center dedicated exclusively to the care of patients with underlying malignancies. Due to the high risk nature of our patient population, antimicrobial usage at our institution is high. Currently, >50% of staphylococci (both methicillin-susceptible and MRSA) isolated at our institution have vancomycin MICs that are ≥1.0 μg/mL (Rolston et al. 2015, 2014b). Our previously published study evaluating the in vitro activity of telavancin, a dual-action lipoglycopeptide, demonstrated potent activity of this agent against most gram-positive pathogens isolated from patients with cancer, including organisms with vancomycin MICs ≥1.0 μg/mL (Rolston et al. 2014b). Many investigators believe that in neutropenic

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http://dx.doi.org/10.1016/j.diagmicrobio.2016.12.010
0732-8893/

patients, there is added value for agents that are bactericidal (Marconescu et al. 2012). Determination of MBCs and comparison of MBC to MIC ratios is one way to evaluate the bactericidal activity of antimicrobial agents. Additionally, time-kill studies are often performed in order to assess bactericidal activity. These additional studies are not performed by clinical laboratories and are generally limited to research settings. In real-time clinical situations, treating physicians have to rely on MIC data. Whether or not MICs, which are in the susceptible range, predict good bactericidal activity is unclear. Other investigators have evaluated the bactericidal activity of telavancin against *S. aureus* isolates with variable results, possibly due to methodologic differences (Barriere et al. 2014; Marconescu et al. 2012; Shaw et al. 2005). None of these studies included isolates from patients with cancer. Thus, we compared the bactericidal activities of vancomycin and telavancin using both methodologies.

2. Materials and methods

2.1. Bacterial isolates and media

Twenty-six clinical MRSA isolates from cancer patients treated at our institution during the years 2013–2014 were obtained for susceptibility testing from the institutional microbiology laboratory. Vancomycin and telavancin MICs and MBCs were performed for these isolates. Four of these isolates were selected for the time-kill experiments based on extremes of their MBC to MIC ratios for vancomycin. Population analysis was conducted on these 4 strains in order to determine whether any of them were heteroresistant to vancomycin, using the methodology described by Wootton et al. (2001). Cation-adjusted Mueller-Hinton broth (CAMHB) by Becton, Dickinson & Co. (Sparks, MD, LOT 4362038) was used for all experiments. Broth was prepared by dissolving 2.2 g of CAMHB in 100-mL water plus 0.002% polysorbate-80 for telavancin according to Clinical and Laboratory Standards Institute guidelines (product # P-4780-100 mL, lot # MKBP5143V) from Sigma-Aldrich (St. Louis, MO). The final cation concentration was calcium (20–25 mg/L) and magnesium (10–12.5 mg/L).

2.2. Antimicrobial susceptibility testing

Telavancin and vancomycin MICs and MBCs were determined using standardized broth microdilution testing as recommended by the CLSI (1999, 2015).

2.3. Time-kill experiments

Vancomycin hydrochloride (Sigma Aldrich, lot # 080M1341V), and telavancin hydrochloride (Theravance Pharmaceuticals, South San Francisco, CA; lot # THR-07-12-01) were used for all experiments. The concentrations used during time-kill experiments represent 4 and 8 times the respective MIC for the individual antimicrobial. Dimethyl sulfoxide (DMSO) (product # D8418-250 mL, lot #SHBC-3313 V) was used for dilution of telavancin according to CLSI procedures. Vancomycin MBC:MIC ratios for the 4 isolates used were 2:1 for 2 isolates and 16:1 for the other 2 isolates. Telavancin MBC:MIC ratios were also 2:1 for the first 2 isolates and 8:1 for the other 2. These represented extreme ranges for both drugs. We chose these divergent MBC:MIC ratios with the rationale that narrower ratios would be associated with better bactericidal activity than wider ratios. Suspensions containing a starting inoculum of approximately 10^7–10^8 CFU/mL were made in polysorbate-80 supplemented (for telavancin only) CAMHB from an overnight growth of each isolate on Mueller-Hinton agar plates at 37 °C. The original isolate suspension was diluted with the polysorbate-80 supplemented CAMHB for a final inoculum of approximately 10^6–10^7 CFU/mL. Vancomycin and telavancin were added at 4 and 8 times the respective MIC values. Cell culture plates containing the antibiotic suspensions were incubated with constant shaking at 37 °C for 24 hours. Samples were taken from each well at 0, 2, 4, 8, and 24 h, and after serial dilution were plated onto blood agar plates. Plated samples were incubated at 37 °C for 24 hours and colony counts were enumerated using the Flash & Grow scanner and software (Neutec Group, Farmingdale, NY). Growth controls containing no antibiotic were sampled, plated, and read as described above. Antibiotic carry-over was assessed by dilution and filtration method, and no carry-over was observed (Huang et al. 2010). All experiments were performed in duplicate and the figures contain graphs of the average data. Additionally, an outside laboratory was used to confirm the time-kill results.

2.4. Analysis of time-kill experiments

The lower limit of bacterial detection utilized was 2.0 $\log_{10}$ CFU/mL. Bacterial concentrations less than 2.0 $\log_{10}$ CFU/mL were counted as 2.0 $\log_{10}$ CFU/mL. Bactericidal effect was defined as a ≥3 $\log_{10}$ decrease in CFU/mL after 24 h compared with the starting inoculum.

3. Results

Of the 26 MRSA isolates tested, all had vancomycin MIC values of ≥1.0 μg/mL, whereas only 1 isolate had a telavancin MIC of 1.0 μg/mL. The bactericidal activity of vancomycin and telavancin against these 26 clinical MRSA isolates as determined by MBC:MIC ratios is shown in Table 1. No tolerance to either agent (defined as an MBC:MIC ratio ≥32:1) was observed. Overall, 85% of isolates had an MBC:MIC ratio ≤4:1 for telavancin, and 65% of isolates had an MBC:MIC ratio ≤4:1 for vancomycin, suggesting somewhat better bactericidal activity for telavancin.

The results of the time-kill studies conducted in our laboratory are shown in Fig. 1 (A–D). Population analysis did not reveal the presence of heteroresistance to vancomycin among any of the 4 isolated tested. Vancomycin demonstrated bactericidal activity (defined as ≥3 log kill over 24 hours) against all 4 isolates. Although telavancin demonstrated steady bacterial killing over a 24-hour period against all 4 isolates, it did not achieve defined bactericidal activity against any. Additionally, the rate of decline (killing) for vancomycin was somewhat greater than that for telavancin for all strains tested. Due to these somewhat unanticipated results, a collaborating laboratory (Anti-infective Research Laboratory of Wayne State University in Detroit, MI) was asked to blindly test the same isolates using time-kill methodology. The results obtained by our partner laboratory are shown in Fig. 1 (E–H). The results for isolate #1 were identical to ours and the results for isolate #4 were very similar. For isolate #1 vancomycin was found to be bactericidal and telavancin was not, by both laboratories. For isolate #4, both vancomycin and telavancin were found to be bactericidal by our partner laboratory, whereas vancomycin achieved defined bactericidal activity, and telavancin came very close (2.98 log reduction over 24 hours) in our hands. There was however, some discordance in the results obtained with the other 2 isolates. Both vancomycin and telavancin failed to achieve killing consistent with bactericidal activity on isolate #2 when tested by our partner laboratory, whereas our laboratory found vancomycin, but not telavancin, to be bactericidal against this strain. Against isolate #3, vancomycin and telavancin were both found to be bactericidal by our partner laboratory, but

Table 1
Bactericidal activity of vancomycin and telavancin against 26 MRSA isolates based on MBC to MIC ratios.

Telavancin			Vancomycin		
MBC:MIC ratio*	No.	(%)	MBC:MIC ratio*	No.	(%)
1:1	1	(4)	1:1	1	(4)
2:1	12	(46)	2:1	12	(46)
4:1	9	(35)	4:1	4	(15)
8:1	3	(11)	8:1	6	(23)
16:1	1	(4)	16:1	3	(12)

No tolerance (defined as an MBC:MIC ratio ≥32:1) was seen for either agent.
* 85% of isolates had an MBC:MIC ratio of ≤4:1 for telavancin and 65% of isolates had an MBC:MIC ratio ≤4:1 for vancomycin.