Influence of the MBC/MIC ratio on the antibacterial activity of vancomycin versus linezolid against methicillin-resistant Staphylococcus aureus isolates in a pharmacodynamic model simulating serum and soft tissue interstitial fluid concentrations reported in diabetic patients

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Objectives: To explore serum and tissue pharmacodynamics of linezolid versus vancomycin against methicillin-resistant Staphylococcus aureus (MRSA) clinical isolates with different MBC/MIC ratios.

Methods: Five strains (vancomycin MIC/MBCs, mg/L) were used: TOL-1 (2/≥64), TOL-2 (1/16), LT-1 and LT-2 (1/8) and NT (1/2). The linezolid MIC/MBC for all strains was 2/≥64 mg/L. A two-compartment dynamic computerized device was used (inocula 10^7 cfu/mL). Free concentrations obtained in serum and interstitial fluid with twice-daily regimens of 1 g of vancomycin or 600 mg of linezolid were simulated over 48 h. ABBCs (differences between control growth curves and killing curves of bacteria exposed to antibiotics; log10 cfu×h/mL) and log10 reductions in initial inocula were calculated.

Results: In serum simulations, vancomycin (AUC₀–₂₄/MIC = 251.8 for TOL-1 and 503.6 for the remaining strains) was bacteriostatic against strains with MBC/MIC ≥8, but bactericidal against NT. In interstitial fluid simulations (AUC₀–₂₄/MIC = 54.6 for TOL-1 and 109.2 for the remaining strains), initial inocula grew in all cases. Linezolid, both in serum (AUC₀–₂₄/MIC = 87.0) and in interstitial fluid (AUC₀–₂₄/MIC = 130.6) simulations, reduced initial inocula ≥2.2 log₁₀ for all strains (apart from LT-1 in serum simulations that showed a bacteriostatic profile). ABBCs were similar in serum and interstitial fluid with linezolid, but significantly lower in interstitial fluid simulations with vancomycin.

Conclusions: From the pharmacodynamic perspective (serum concentrations), vancomycin tolerance should include MBC/MIC ≥8 since strains exhibiting this ratio showed bacteriostatic profiles similar to those obtained with isolates with MBC/MIC ratios of 16 or 32. Insufficient concentrations of vancomycin at the simulated infected site were linked to bacteriological failure. Free concentrations of linezolid at the infection site pharmacodynamically covered MRSA.

Keywords: MRSA, tolerance, pharmacodynamic simulations, diabetic foot infections

Introduction

A multicentre study testing Staphylococcus aureus isolates from patients with skin/soft tissue infections attended in emergency departments in the USA showed that 59% of isolates were methicillin-resistant S. aureus (MRSA).¹ Vancomycin tolerance, clustered in MRSA isolates with vancomycin MICs of 1–2 mg/L,² is ‘invisible’ to clinicians since MBC is not routinely determined. In a previous study, the tolerance rate reported among MRSA isolated from bloodstream infections was 20.1% (using as criterion MBC/MIC ≥32) or 24.8% (MBC/MIC ≥16), with an additional 6.2% of strains exhibiting an MBC/MIC ratio of 8.³ In skin/soft tissue infections, as in diabetic foot infections, impaired antibiotic penetration compromises antimicrobial activity.⁴ Thus, pharmacokinetic/pharmacodynamic evaluation should be based on tissue rather than serum concentrations. Penetration of vancomycin into tissues is variable, being lower in diabetic patients.⁵ In contrast, linezolid shows good penetration in inflamed tissues.⁶
This study explores serum and tissue pharmacodynamics of linezolid versus vancomycin against vancomycin-susceptible MRSA clinical isolates with different values of MBC/MIC ratio.

Methods

Strains

Five MRSA clinical isolates from diabetic patients with foot infections were selected based on their vancomycin MIC/MBC ratio. Two strains, TOL-1 (MIC/MBC = 2/64 mg/L) and TOL-2 (MIC/MBC = 1/16 mg/L), were tolerant (MIC/MIC ratio ≥16), two strains, LT-1 and LT-2 (both with MIC/MBC = 1/8 mg/L), exhibited an MBC/MIC ratio of 8 and one strain had a ratio of 2 (strain NT; MIC/MBC = 1/2 mg/L). None of the strains showed heteroresistance by the modified population analysis profile method.

In vitro kinetic system

The two-compartment system used was previously described in detail. MRSA strains were exposed to simulated free concentrations in serum and soft tissue interstitial fluid reported in diabetic patients after multiple doses of 1 g of vancomycin intravenously twice daily or 600 mg of linezolid orally twice daily over 48 h. Reported total concentrations for vancomycin in serum (C_max = 40 mg/L and C_min = 10 mg/L) were used to calculate target free concentrations in serum (fC_max = 20 mg/L, fC_min = 5 mg/L and t_{1/2} = 6 h), assuming 50% protein binding. For linezolid, reported total C_max = 14.5 mg/L, C_min = 2 mg/L and AUC_0–12 = 114.0 mg·h/L were used to calculate target free concentrations in serum (fC_max = 10.0 mg/L, fAUC_0–12 = 78.7 mg·h/L and t_{1/2} = 5.5 h) using a protein binding rate of 31%. To calculate target interstitial fluid concentrations, the median free tissue/free serum concentration ratio in diabetic patients of 0.1 was assumed for vancomycin and the fAUC_0–12 in tissue/fAUC_0–12 in serum ratio of 1.30 in diabetics was assumed for linezolid. Drugs were injected into the central reservoir using a syringe pump (Gilson SA, Villiers-le-Bel, France) to achieve peak concentrations desired at 0 h (vancomycin) or 2 h (linezolid). The mono-exponential concentration decay in the central compartment was achieved by a continuous dilution–elimination process using computerized peristaltic pumps (Masterflex, Cole-Parmer Instrument Co., Chicago, IL, USA) set to simulate the t_{1/2} of the antimicrobials. Bacteria were inoculated and confined to the extracapillary space of the dialyzer unit (Fresenius Medical Care S.A., Barcelona, Spain) representing the peripheral compartment. Final initial inocula of 1 · 10^7 cfu/mL were used and exposed to fluctuating drug concentrations. The high surface-area-to-volume ratio of the dialysis unit (∼200 cm^2/mL) yielded a rapid drug redistribution between the central and the peripheral compartment. The entire system was maintained at 37°C throughout the simulation. Control drug-free simulations were only performed in parallel. Each experiment was run in triplicate.

Pharmacokinetic and pharmacodynamic analysis

Samples were collected at 0, 2, 4, 6, 8 and 12 h from the peripheral compartment to determine concentrations by bioassay using S. aureus ATCC 29213 (for linezolid) and Bacillus subtilis ATCC 6533 (for vancomycin). The assay was linear (0.998–0.997) over the range tested, with detection limits of 1 and 0.12 mg/L, respectively. Inter- and intra-day coefficients of variation were always <5%. Experimental t_{1/2}, AUC_0–12, fC_max and fC_min were determined using the WinNonlin program (version 5.3 Pharsight, Mountain View, CA, USA).

Samples (0.5 mL) collected from the peripheral compartment over 48 h were serially diluted and plated onto Mueller–Hinton agar (Difco Laboratories, Detroit, MI, USA) using a spiral plater (Don Whitley, Shipley, UK) for viable cell quantification. The level of detection was 50 cfu/mL.

Antibacterial effects were studied by calculating log_{10} cfu/mL reductions between time 0 and 24 or 48 h, and by determining the difference between the trapezoidal area under the bacterial count–time curve in the absence (growth curve) and presence (killing curve) of the antibiotic from time 0 to 48 h (ABBC; log_{10} cfu·h/mL).

Statistical analysis

An unpaired t-test or one-way ANOVA with the Tukey post test was used to compare serum versus interstitial fluid ABBCs, and for comparison of activity between strains and between antibiotics in serum and interstitial fluid. P < 0.01 was considered significant.

Results

Figure 1 includes experimentally determined versus target concentrations and pharmacokinetic parameters of vancomycin and linezolid in serum and interstitial fluid. Experimental concentrations were within 10% of target values. At all sampling times, concentrations in interstitial fluid were significantly lower than those in serum for vancomycin and significantly higher for linezolid. Table 1 shows fAUC_0–24/MIC values.

Figure 1 also shows mean bacterial counts in antibiotic and antibiotic-free simulations. In serum simulations, vancomycin was bacteriostatic against the four strains with MBC/MIC ratios ≥8 but bactericidal against strain NT, the log_{10} reductions in initial inocula between this strain and the remaining study strains being significantly different at 48 h (Table 1). In interstitial fluid simulations with vancomycin, initial inocula increased at 24 and 48 h for all strains. In linezolid simulations, initial inocula reductions of ≥2.2 log_{10} in serum (for all strains apart from LT-1) and ≥2.5 log_{10} in interstitial fluid (for all strains) were found at 48 h.

Figure 1 also shows ABBCs in serum and interstitial fluid simulations. For vancomycin, serum ABBCs were significantly higher than interstitial fluid ABBCs for all strains, whereas for linezolid interstitial fluid ABBCs were higher than serum ABBCs, although differences were only significant for TOL-1 and LT-1 strains. When comparing vancomycin with linezolid, significantly higher serum ABBCs were found for vancomycin only for the NT isolate. In contrast, interstitial fluid ABBCs for linezolid were significantly higher than those for vancomycin for all strains.

Discussion

The 22% prevalence of isolates with an MBC/MIC ratio of 8–16 among MRSA isolates from diabetic patients found in our laboratory was the basis for the present study exploring the influence of MBC/MIC ratio on antibacterial activity. All study strains were susceptible to study drugs according to CLSI breakpoints, as were determined using the WinNonlin program (version 5.3 Pharsight, Mountain View, CA, USA).

Figure 1. Concentration profiles obtained with the vancomycin (a) and linezolid (b) regimens tested. Target profile (broken lines) versus experimentally measured concentrations (continuous lines) in serum (filled symbols) and in soft tissue interstitial fluid (open symbols). Log_{10} colony counts over time for growth curves (G-C, broken grey lines) (c and d) for serum simulations with vancomycin (c) and linezolid (d) and for interstitial fluid simulations with vancomycin (e) and linezolid (f). Serum and interstitial fluid ABBCs for vancomycin (g) and linezolid (h). i. fluid, interstitial fluid.
The target for clinical effectiveness (tions in diabetic patients. Values of serum AUC/MIC of vancomycin have been classically described as a virulence factor in soft tissue infec-
<table>
<thead>
<tr>
<th>Isolate</th>
<th>fAUC\textsubscript{0–24}/MIC (AUC\textsubscript{0–24}/MIC)</th>
<th>Log\textsubscript{10} cfu/mL change in viable counts</th>
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<td></td>
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<td>24 h</td>
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<td>serum</td>
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<tr>
<td>TOL-1</td>
<td>125.9 (251.8)</td>
<td>0.4 ± 0.6</td>
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<tr>
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<td>251.8 (503.6)</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>LT-1</td>
<td>251.8 (503.6)</td>
<td>0.7 ± 0.7</td>
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<tr>
<td>LT-2</td>
<td>251.8 (503.6)</td>
<td>1.1 ± 0.6</td>
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<tr>
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<td>251.8 (503.6)</td>
<td>2.8 ± 0.3</td>
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<tr>
<td>TOL-1</td>
<td>27.3 (54.6)</td>
<td>−1.5 ± 0.1\textsuperscript{b}</td>
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<tr>
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<td>NT</td>
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<td>TOL-1</td>
<td>66.4 (87.0)</td>
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<td>LT-2</td>
<td>99.7 (130.6)</td>
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</tr>
<tr>
<td>NT</td>
<td>99.7 (130.6)</td>
<td>2.3 ± 0.5\textsuperscript{d}</td>
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Negative values indicate regrowth of the initial inoculum.
\textsuperscript{a}P < 0.01 versus values for the NT strain.
\textsuperscript{b}P < 0.01 versus values in serum.
\textsuperscript{c}P < 0.01 versus remaining strains.
\textsuperscript{d}P < 0.01 versus vancomycin.

usual, since resistance to vancomycin or linezolid is exceptionally rare in MRSA. High inocula were used since inoculum size has been classically described as a virulence factor in soft tissue infec-
tions in diabetic patients. Values of serum AUC/MIC of vancomycin obtained for all strains (except TOL-1) were above those reported as the target for clinical effectiveness (≥400).12 However, although a strict definition of tolerance (MBC/MIC ratio ≥32 or ≥16 when vancomycin MBC is ≥32 mg/L)13,14 only applied to strain TOL-1, vancomycin was only bactericidal against strain NT and bacterio-
static against strains with MBC/MIC ratio ≥8, without differences in activity against strain TOL-1 and strains with an MBC/MIC ratio of 16 or 8. Therefore, from the pharmacodynamic perspective, the concept of vancomycin tolerance could be widened by including strains with MBC/MIC ratios low as 8. Due to the low vancomycin penetration in soft tissue/interstitial fluid,2 activity was less in inter-
stitial fluid simulations, with regrowth in the initial inocula for all

strains, regardless of tolerance or MBC/MIC ratio, linked to low
AUC/MIC values (54.6 for TOL-1 and 109.2 for the remaining
strains). It has been suggested that even the new CLSI breakpoint for vancomycin (MIC <2 mg/L) could not precisely discriminate cases that will respond to therapy from those that will not,15,16 and that the breakpoint should be lowered to ≤1 mg/L or even ≤0.5 mg/L.17 The results of this study support this suggestion since all strains were susceptible and no activity was obtained in the simulated infection site where initial inocula increased.

In the case of linezolid, the value of AUC/MIC in serum was higher than that required for efficacy in a previous animal model,18 and initial inocula reductions were ≥2.2 log\textsubscript{10} for all strains except LT-1 (showing bacteriostasis). Due to the high linezolo-
id penetration in interstitial fluid, AUC/MIC was 130.6 (AUC/
MIC= 99.7), a value similar to the proposed cut-off for efficacy.19 In our study, the linezolid MBC/MIC ratio was ≥32 for all strains, as previously reported for 80% of MRSA strains,20 but tolerance had no implications for antibacterial activity since in interstitial fluid simulations initial inocula reductions at 48 h were ≥2.5 log\textsubscript{10} for all strains.

The ABBC profile was similar in serum and interstitial fluid for linezolid, thus offering an advantage over vancomycin, which presented different profiles due to the unacceptable, low ABBCs in the simulated infection site.

The results of this study show that (i) from the pharmacody-
namic perspective (serum simulations) the concept of vancomycin tolerance should include an MBC/MIC ratio of ≥8, since vancomycin was bacteriostatic against strains with this ratio without differences in colony counts at 24h and 48h between these strains and those with MBC/MIC ratio of ≥16, (ii) insufficient concentrations of vancomycin at the simulated infection site were linked to bacteriological failure, not decreasing the initial bacterial inocula, and (iii) free concentrations of linezolid at the infection site were sufficient to pharmacodynamically cover MRSA.

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**Transparency declarations**
None to declare.

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