Microbiological effects of prior vancomycin use in patients with methicillin-resistant Staphylococcus aureus bacteraemia

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Received 10 September 2007; returned 27 September 2007; revised 18 October 2007; accepted 19 October 2007

Background: We sought to determine whether prior vancomycin use (within 30 days) in patients who develop methicillin-resistant Staphylococcus aureus (MRSA) bacteraemia is associated with isolates of reduced vancomycin susceptibility and killing in vitro.

Methods: Thirty-eight MRSA from previously vancomycin-treated patients and 43 MRSA from vancomycin-naive patients were evaluated by vancomycin and daptomycin CLSI broth microdilution and killing assays. PCR was used to determine accessory gene regulator (agr) type and staphylococcal cassette chromosome mec (SCCmec) type, and nucleotide sequencing was used to determine spa type.

Results: Vancomycin MICs were 0.5, 1.0 and 2.0 mg/L for 19, 55 and 7 isolates, respectively. Daptomycin MICs were 0.25, 0.5, 1.0 and 2.0 mg/L for 4, 50, 26 and 1 isolate, respectively. The agr-type distribution was agr group II (59%), group I (25%) and group III (16%); 90% harboured SCCmec II. The genetic background extrapolated by spa-typing showed that 58% of the isolates were of clonal complex 5. MRSA bloodstream isolates from patients who had received vancomycin within the preceding 30 days had a significantly decreased vancomycin killing at 24 h in vitro (median log10 decrease, 3.1 versus 2.2 cfu/mL; P = 0.021) and a significantly higher vancomycin MIC than isolates obtained from patients without that history (P = 0.002).

Conclusions: MRSA bloodstream isolates from patients recently treated with vancomycin may demonstrate reduced susceptibility and increased tolerance to vancomycin in vitro. Given that such microbiological phenotypes have been associated with reduced vancomycin efficacy, consideration may be given to alternative Gram-positive antimicrobial therapy in patients who have recently been treated with vancomycin.

Keywords: glycopeptides, daptomycin, MRSA, resistance

Introduction

Staphylococcus aureus is a significant cause of nosocomial bloodstream infections and now represents the most common aetiological agent in bacterial endocarditis in developed countries.1 Currently, over 60% of clinical S. aureus isolates from intensive care units (ICUs) in the USA and over 50% of isolates from inpatient non-ICU hospital settings are caused by methicillin-resistant S. aureus (MRSA).2,3 Although vancomycin has been standard therapy for the treatment of MRSA bacteraemia, treatment response may be suboptimal.4-7 Recent data suggest an increased risk for vancomycin treatment failure in bacteraemia caused by susceptible

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MRSA that demonstrate vancomycin MIC of >1 mg/L, decreasing vancomycin killing in vitro, and which belong to specific genotypes.8–12

Daptomycin, a lipopeptide antimicrobial agent that was recently approved for the treatment of MRSA bacteremia, binds to the bacterial cell membrane, causing bacterial cell death via an efflux of potassium ions.13 Given that vancomycin exerts its antimicrobial activity through a different mechanism, exposure to vancomycin is not expected to affect daptomycin susceptibility.

A number of studies have examined the effect of previous antibiotic use on antibiotic resistance,14–16 but the effects of previous vancomycin use in patients with MRSA bacteremia have not been examined. The primary objective of this study was to determine whether prior administration of vancomycin within 30 days in vivo was associated with the development of bacteremia with MRSA demonstrating reduced vancomycin and daptomycin susceptibility (MIC). Secondary objectives were to examine the relationship of prior vancomycin use to vancomycin and daptomycin bactericidal activity and accessory gene regulator (agr) function. Given the previous observations linking S. aureus genetic background as defined by agr genotype and vancomycin treatment outcome,9 we have determined this genotype for both vancomycin-exposed and -naive isolates. Furthermore, with the emergence of community-associated MRSA strains, with the appreciation that these genetic background differences may confound vancomycin exposure and consequent vancomycin susceptibility differences in these strains, we performed staphylococcal cassette chromosome mec (SCCmeC) type and staphylococcal protein A (spa) typing for all the evaluated MRSA in this study.

Methods

Patients

From a pool of prospectively collected MRSA isolates, 81 MRSA bloodstream isolates from 81 different patients were arbitrarily selected. These 81 patients were previously enrolled in prospective Phase III/IV trials between July 1998 and March 2003 from 24 hospitals representing 16 states (CT, DE, FL, GA, HI, IA, IL, LA, MA, MD, NJ, NY, OH, PA, TX and WA). For each study, appropriate ethical regulations were followed, and the study was approved by the Ethics Committee or institutional review board at each participating institution.

Definitions

Patient cases were categorized by vancomycin exposure in the 30 days prior to the development of MRSA bacteremia. The vancomycin-use patients were defined as patients who received at least one dose of intravenous vancomycin within 30 days prior to the initial MRSA positive blood culture. The ‘no known vancomycin use’ group included those patients who had no known vancomycin use within 30 days prior to the initial MRSA positive blood culture. Vancomycin use during the 30 days prior to presentation with MRSA bacteremia was identified by chart review, by patient interview and by discussion with the patient’s primary care physician and other physicians identified as having provided care during that time period. Patients were classified as having a clinically significant MRSA infection, as defined elsewhere.17

Microbiological testing

Isolates were stored at −70°C and were grown and maintained on trypticase-soy blood agar for subsequent testing. Blinded from the clinical data, daptomycin and vancomycin susceptibility testing was performed using CLSI microbroth dilution.18 Cation-adjusted Mueller–Hinton broth supplemented to contain calcium at a final concentration of 50–55 mg/L was used for daptomycin susceptibility testing. In vitro daptomycin killing assays (4 h) were performed in similar media containing 8 mg/L daptomycin using an initial bacterial inoculum of 107–108 cfu/mL. Vancomycin killing assays (24 h) were performed using an initial bacterial inoculum of 107–109 cfu/mL in Mueller–Hinton broth containing 16 mg/L vancomycin.

PCR was used to determine agr type and SCCmeC type, and nucleotide sequencing was used to determine spa type, as described previously.19–22 A semi-quantitative assessment of agr function was performed using the expression of δ-haemolysin activity on sheep blood agar, as described previously.23

To decrease bias, clinical data were documented before microbiological testing was performed. Investigators who performed in vitro susceptibility and killing assays, and molecular typing, were blinded to all clinical information, including prior vancomycin therapy.

Statistics

Ordinal and non-parametric continuous variables were compared using Kruskal–Wallis analysis of variance (ANOVA). Categorical variables were compared using the χ2 or Fisher’s exact test where appropriate. Spearman’s correlation was used to examine the univariate relationship between daptomycin and vancomycin MICs. Logistic regression, using backward stepping, was used for multivariate analysis of risk factors for vancomycin MICs of 2.0 mg/L. All statistical procedures were performed with Systat 11 (Systat Software Inc., Point Richmond, CA, USA) or SPSS 13.0 (SPSS Inc., Chicago, IL, USA).

Results

Patient demographics

We studied MRSA isolates from 81 patients with bacteremia that occurred from July 1998 to March 2003, in which 38 patients had received intravenous vancomycin therapy within 30 days prior to the MRSA infection (vancomycin use group) and 43 had no known intravenous vancomycin administration during that time (no known vancomycin use group). Of the 38 patients who received vancomycin, 9 (24%) received a single dose and 76% (29/38) received more than one vancomycin dose. Fourteen of the 38 patients (37%) received vancomycin within 7 days of MRSA isolation, and 63% (24/38) received no vancomycin within the week prior to MRSA isolation. The indications for antecedent vancomycin were as follows: 10 patients received vancomycin empirically; 9 received vancomycin perioperatively, 1 patient received 3 days of vancomycin empirically until the causative organism was identified as vancomycin-resistant enterococci and the drug was discontinued; and 28 patients received vancomycin for MRSA infection. Patient age ranged from 24 to 99 years, with a mean age (± SD) of 65.8 ± 15.7 years, 57% were male, and 43% were in an ICU at the onset of the infection. We noted the vancomycin use group...
to be enriched for haemodialysis patients when compared with the group with no known vancomycin use (Table 1).

Organism characteristics

All MRSA were susceptible to vancomycin (MIC ≤ 2.0 mg/L), and 99% (80/81) were susceptible to daptomycin (MIC ≤ 1.0 mg/L). Isolates were more likely to be agr group II (59%), followed by group I (25%) and group III (16%). The majority (90%; 73/81) of the MRSA were SCCmec II. Eight (10%) were SCCmec IV, and none were SCCmec I or III. spa-typing was performed for 77 of the isolates and showed that 58% (45/77) were predicted to be of clonal complex 5. No differences in agr type, SCCmec type and spa type were noted for the vancomycin use group when compared with the no known vancomycin use group. A trend was noted between prior vancomycin use and decreased agr expression as determined by d-haemolysin activity (67% versus 84%, P = 0.081) (Table 2).

Table 1. Clinical characteristics of 81 patients with MRSA bacteraemia and effects of antecedent vancomycin use

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No-VAN use (n = 43)</th>
<th>VAN use (n = 38)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years, mean ± SD (median)</td>
<td>65 ± 16 (69)</td>
<td>67 ± 15 (70)</td>
<td>0.906</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>24 (56)</td>
<td>22 (58)</td>
<td>0.850</td>
</tr>
<tr>
<td>CL\textsubscript{CR} in mL/min, mean ± SD (median)</td>
<td>75 ± 11 (66)</td>
<td>64 ± 36 (60)</td>
<td>0.215</td>
</tr>
<tr>
<td>Institution associated with acquisition, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hospital</td>
<td>37 (86)</td>
<td>27 (71)</td>
<td>0.098</td>
</tr>
<tr>
<td>nursing home</td>
<td>4 (9)</td>
<td>4 (11)</td>
<td>0.854</td>
</tr>
<tr>
<td>ICU patient on day 1 of MRSA, n (%)</td>
<td>22 (51)</td>
<td>13 (34)</td>
<td>0.124</td>
</tr>
<tr>
<td>Underlying condition, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>heart disease</td>
<td>34 (79)</td>
<td>26 (68)</td>
<td>0.275</td>
</tr>
<tr>
<td>lung disease</td>
<td>11 (26)</td>
<td>11 (29)</td>
<td>0.734</td>
</tr>
<tr>
<td>ESRD on HD</td>
<td>1 (2)</td>
<td>9 (24)</td>
<td>0.004</td>
</tr>
<tr>
<td>malignancy</td>
<td>4 (9)</td>
<td>8 (21)</td>
<td>0.137</td>
</tr>
<tr>
<td>diabetes mellitus</td>
<td>14 (33)</td>
<td>11 (29)</td>
<td>0.726</td>
</tr>
<tr>
<td>alcohol abuse</td>
<td>5 (7)</td>
<td>1 (3)</td>
<td>0.368</td>
</tr>
</tbody>
</table>

CL\textsubscript{CR}, creatinine clearance; ESRD, endstage renal disease; HD, haemodialysis; ICU patient, patient in an ICU at the time of baseline diagnosis; no-VAN use, patients who had no known prior use (within 30 days) of intravenous vancomycin; VAN use, patients who had previously received (within 30 days) intravenous vancomycin.

Table 2. Phenotype of MRSA isolates from 81 patients with MRSA bacteraemia and effects of antecedent vancomycin use

<table>
<thead>
<tr>
<th>Organism phenotype</th>
<th>No-VAN use (n = 43)</th>
<th>VAN use (n = 38)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAN MIC (mg/L), n (%)</td>
<td></td>
<td></td>
<td>0.002</td>
</tr>
<tr>
<td>0.5</td>
<td>15 (35)</td>
<td>4 (11)</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>27 (63)</td>
<td>28 (74)</td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>1 (2)</td>
<td>6 (16)</td>
<td></td>
</tr>
<tr>
<td>DAP MIC (mg/L), n (%)</td>
<td></td>
<td></td>
<td>0.111</td>
</tr>
<tr>
<td>0.25</td>
<td>3 (7)</td>
<td>1 (3)</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>29 (67)</td>
<td>21 (55)</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>10 (23)</td>
<td>16 (42)</td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>1 (2)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>VAN-log\textsubscript{10} #, mean ± SD (median)</td>
<td>3.15 ± 0.95 (3.08)</td>
<td>2.28 ± 0.81 (2.17)</td>
<td>0.021</td>
</tr>
<tr>
<td>DAP-log\textsubscript{10} #, mean ± SD (median)</td>
<td>3.81 ± 0.76 (3.99)</td>
<td>3.82 ± 0.88 (4.02)</td>
<td>0.932</td>
</tr>
<tr>
<td>Lack of d-lysin production, n (%)</td>
<td>29 (67)</td>
<td>32 (84)</td>
<td>0.081</td>
</tr>
</tbody>
</table>

No-VAN use, patients who had no known prior use (within 30 days) of intravenous vancomycin; VAN use, patients who had previously received (within 30 days) intravenous vancomycin; DAP, daptomycin; VAN, vancomycin.

Log\textsubscript{10} killing reported is the change in cfu/mL at 4 h for daptomycin and at 24 h for vancomycin.

Lack of d-lysin production was used as a marker for the decrease in the expression of agr.

P values for VAN MIC and DAP MIC are from the Kruskal–Wallis ANOVA and represent the ordinal relationship between MIC values in the no-VAN use and the VAN use groups. The Mann–Whitney U-test was used to compare the VAN-log\textsubscript{10} \# and the DAP-log\textsubscript{10} \# between the two groups (no-VAN use and VAN use), and \( x^2 \) was used to compare the lack of d-lysin production between the no-VAN use and VAN use groups.
Vancomycin use and vancomycin and daptomycin susceptibility

Vancomycin MICs were significantly higher in patients with antecedent vancomycin use, according to the univariate analysis (P = 0.002 by Kruskal–Wallis ANOVA) (Table 2). In addition, multivariate analysis (logistic regression) found patients with previous vancomycin use to be more likely to have MRSA with vancomycin MICs of 1.0–2.0 mg/L (OR 4.55; 95% CI, 1.36–15.4; P = 0.014). No other studied clinical or microbiological characteristics were significantly associated with vancomycin MICs of 1.0–2.0 mg/L.

A trend to higher daptomycin MIC values, although not statistically significant, was noted for the prior vancomycin use group (P = 0.111) by Kruskal–Wallis ANOVA) (Table 2). However, in reviewing our 81 clinical MRSA isolates, we noted that a relationship between vancomycin and daptomycin MICs may exist (Spearman’s ρ = 0.285, P = 0.01). Daptomycin MICs were 0.25, 0.5, 1.0 and 2.0 mg/L in 16%, 63%, 21% and 0%, respectively, of the 19 MRSA, with vancomycin MICs of 0.5 mg/L. Daptomycin MICs were 0.25, 0.5, 1.0 and 2.0 mg/L in 2%, 65%, 31% and 2% of MRSA, respectively, when vancomycin MICs were 1.0 mg/L. In addition, of the seven MRSA with vancomycin MICs of 2.0 mg/L, two had daptomycin MICs of 0.5 mg/L and five had daptomycin MICs of 1.0 mg/L.

Relationship between prior vancomycin use and vancomycin and daptomycin in vitro bactericidal activity

Prior vancomycin use in vivo was significantly associated with decreased vancomycin killing at 24 h in vitro, with a median vancomycin log₁₀ cfu of killing/mL of 3.08 in those with no known vancomycin use compared with 2.16 for those with previous vancomycin use (P = 0.021). However, we noted no differences in daptomycin killing of MRSA at 4 h in vitro obtained from patients with no known vancomycin use and from those with previous vancomycin use (P = 0.932) (Table 2). Daptomycin retained bactericidal activity in both groups.

Discussion

The initiation of prompt antimicrobial therapy with activity against the offending pathogen has been associated with improved clinical outcomes in nosocomial infection. Previous exposure to antimicrobials is a risk factor for multidrug-resistant pathogens. In the cases of nosocomial pneumonia that develop during or shortly following antibiotic therapy, recommendations by the American Thoracic Society and Infectious Diseases Society of America call for empirical antimicrobial therapy different than the initial class. Given the hypothesis that vancomycin exposure has the potential to select for MRSA with reduced susceptibility and increased tolerance to glycopeptides, we evaluated the in vitro activity of vancomycin against clinical MRSA bloodstream isolates based on whether patients had received any vancomycin in the 30 days prior to the onset of MRSA bacteraemia.

We evaluated 38 MRSA from patients who had received vancomycin and compared them with 43 MRSA from vancomycin-untreated patients. Consistent with our previous findings on the molecular epidemiology of MRSA bacteraemia, 60% of the isolates were agr group II, with a similar agr group distribution between vancomycin-treated and untreated patients. The vast majority of MRSA in both groups harboured SCCmec type II, consistent with nosocomial-based strains as was noted during 1998–2003 prior to the surge in community-based SCCmec IV-harbouring MRSA strains.

We found that MRSA from patients who had received vancomycin within the previous 30 days demonstrated significantly higher vancomycin MICs and reduced vancomycin killing in vitro than MRSA from vancomycin-untreated patients. Published data have demonstrated that infections caused by MRSA with reduced susceptibility to vancomycin and demonstrating decreased vancomycin killing in vitro are associated with inferior clinical efficacy of vancomycin. One study showed that the clinical success of vancomycin in the treatment of MRSA bacteraemia falls from 56% to under 10% when the vancomycin MIC increased from 0.5 to 1.0–2.0 mg/L.8 In the same study, decreased killing by vancomycin in vitro was associated with higher likelihood of treatment failure. This association of decreased in vitro killing with decreased clinical efficacy of vancomycin in the treatment of MRSA bacteraemia has been reproduced.12 In a more recent study comparing 51 patients with a variety of infections caused by MRSA (vancomycin MIC 2 mg/L) with 44 patients (MIC <2 mg/L), response was significantly lower (62% versus 85%, P = 0.02) and infection-related mortality was higher (24% versus 10%) in the high MIC group.11 In addition, a high MIC of vancomycin was an independent predictor of poor response in the multivariate analysis of these MRSA infections.11 Combining these prior data with the findings of this study, antecedent vancomycin exposure is expected to diminish the therapeutic efficacy of vancomycin. Although this makes intuitive sense, further study is warranted to strengthen this argument. Such an evaluation would be important as it would potentially advocate alternative empirical or targeted therapy against MRSA in patients who have been previously treated with vancomycin.

A survival advantage is likely conferred to S. aureus under vancomycin selection pressure with a decrease or loss of agr function, with loss of function mutations being identified in clinical bloodstream MRSA after vancomycin exposure accompanying the development of vancomycin heteroresistance.23,26 Consistent with these data, we found that a clinical history of vancomycin use within 30 days was accompanied by a trend of decreased expression of agr. The effects of suppression of agr function by vancomycin exposure are expected to influence not only antimicrobial resistance, but also bacterial virulence properties such as resistance to platelet microbicidal proteins (PMPs), secreted by platelets at the sites of endothelial injury. Prior studies have shown PMP resistance to develop with exposure to vancomycin both in vitro and in vivo. Resistance to PMPs is associated with prolonged and complicated bacteraemia, with increased risk in developing metastatic foci of infection and endocarditis.27 Whether patients previously treated with vancomycin were more likely to have isolates harbouring virulence traits selected by vancomycin that would more likely select for bacteraemia relapse would be a subject of great interest.

Several investigators have noted an in vitro relationship between vancomycin and daptomycin susceptibility in S. aureus, with isolates showing higher vancomycin MICs to also demonstrate higher daptomycin MICs.28,29 Furthermore, recent whole-genome sequencing has detected the development of
mutations in the genes rpoC and yycH, previously associated with reduced daptomycin susceptibility in S. aureus, to occur with vancomycin therapy.20 We noted a shift towards increased daptomycin MICs in patients who were previously exposed to vancomycin, compared with those who were not exposed to vancomycin. However, we did not find any significant reduction in the in vitro bactericidal activity of daptomycin in MRSA from vancomycin-treated patients. Although there clearly appears to be an in vitro link between vancomycin and daptomycin susceptibility, the clinical significance remains to be determined. Given the reduced efficacy of vancomycin seen in the treatment of MRSA with vancomycin MICs at the upper limit of the susceptible range, clinical studies establishing superior alternative therapeutics would be important.

The enrichment of the vancomycin-exposed population for haemodialysis patients compared with the vancomycin-naive population is not surprising given the low threshold of clinicians treating these patients in using this drug, both because of their risk of systemic MRSA infections and the ease of administration. Clinicians selecting empirical therapy for MRSA infections in these patients should be aware of the decreased susceptibility that results from vancomycin exposure and the appreciation of decreased vancomycin efficacy that is associated with these less-susceptible strains. This finding may also have considerable clinical trial design implications for studies aimed at evaluating MRSA treatment strategies, particularly when trying to evaluate the ‘high-risk’ patients for vancomycin-treatment failure, such as those with vancomycin MIC >1 mg/L. Such studies would likely select for these less-susceptible MRSA isolates when they include haemodialysis patients.

In conclusion, patients with MRSA bacteraemia who received vancomycin within 30 days prior to bacteraemia yielded MRSA isolates with higher vancomycin MICs and reduced vancomycin killing in vitro. A trend to decreased agr activity and increased daptomycin MICs was also noted in patients with a clinical history of vancomycin use. Future studies are required to define the clinical significance of these microbiological observations in order to establish the role of the novel Gram-positive antimicrobial agents in the treatment of MRSA infections, particularly in settings where the vancomycin MIC is at the upper limit of microbiological susceptibility.

Acknowledgements

We thank Jerome Schentag’s Lab for efforts in identifying, re-isolating and preparing all the isolates for shipping.

Funding

This project was funded through an intermural grant from New York Medical College, Valhalla, NY. D. A. R. is supported in part by grants from the American Heart Association and the National Institute of General Medical Sciences (GM080602). G. S. is receiving research funding from Cubist Pharmaceuticals (Lexington, MA, USA). P. A. M. is receiving research funding from Pfizer Pharmaceuticals (New York, NY, USA).

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Transparency declarations

P. A. M. is a member of the speakers’ bureau for Cubist and Pfizer. A. F. has received research funding from Theravance and Wyeth Pharmaceuticals. G. S. has received research funding from Cubist, has been a consultant for Cubist and Pfizer, and has been on the speakers’ bureau for Cubist, Merck, Pfizer and Wyeth. All other authors: no conflicts.

References


