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EXPERIMENTAL THERAPEUTICS

AUC/MIC Pharmacodynamic Target Is Not a Good Predictor of Vancomycin Efficacy in Methicillin-Resistant *Staphylococcus aureus* Experimental Endocarditis

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ABSTRACT The aim of this *in vivo* study was to compare the efficacy of vancomycin at standard doses (VAN-SD) to that of VAN at adjusted doses (VAN-AD) in achieving a VAN area under the curve/MIC ratio (AUC/MIC) of ≥400 against three methicillin-resistant *Staphylococcus aureus* (MRSA) strains with different microdilution VAN MICs in an experimental endocarditis model. The valve vegetation bacterial counts after 48 h of VAN therapy were compared, and no differences were observed between the two treatment groups for any of the three strains tested. Overall, for VAN-SD and VAN-AD, the rates of sterile vegetations were 15/45 (33.3%) and 21/49 (42.8%) ($P = 0.343$), while the medians (interquartile ranges [IQRs]) for $\log_{10}$ CFU/g of vegetation were 2 (0 to 6.9) and 2 (0 to 4.5) ($P = 0.384$), respectively. In conclusion, this VAN AUC/MIC pharmacodynamic target was not a good predictor of vancomycin efficacy in MRSA experimental endocarditis.

KEYWORDS *Staphylococcus aureus*, MRSA, experimental endocarditis, vancomycin MIC, pharmacodynamic target

Despite the high rates of treatment failure described in the literature, vancomycin is still recommended against methicillin-resistant *Staphylococcus aureus* (MRSA) in infective endocarditis (1–3). To date, no alternative antibiotic or combination with vancomycin has demonstrated a superior effectiveness with respect to vancomycin in MRSA endocarditis in a clinical trial (4–6).Vancomycin failure in infective endocarditis has been attributed to limited *in vitro* killing activity against *S. aureus* (7) and poor penetration of cardiac vegetations (8–10).

The area under the curve/MIC ratio (AUC/MIC) has been identified as the best predictor of vancomycin activity against *S. aureus* (11). Achieving an AUC/MIC equal to or higher than 400 or a minimum blood plasma concentration (C_{min}) of 20 mg/liter during vancomycin therapy has been associated with better clinical responses to vancomycin in *S. aureus* lower respiratory tract infections and bacteremia (12, 13). However, these pharmacodynamic indexes have not been validated for infective endocarditis. In addition, the increased risk of nephrotoxicity and ototoxicity associated

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TABLE 1 Pharmacodynamic profile and dosage regimens for the three strains obtained from patients admitted to the Hospital Clinic in Barcelona, Spain, and diagnosed with infective endocarditis[a]

Treatment group for strain	VAN MIC (mg/liter) by Etest/MD	C_{max}/C_{min} (mg/liter)	AUC/Etest MIC	AUC/MD MIC
MRSA-196	0.5/0.5			
VAN-SD (1 g/12 h)		56/6	299/0.5 = 598	299/0.5 = 598
VAN-AD (1.25 g/8 h)		96/17	621/0.5 = 1,242	621/0.5 = 1,242
MRSA-572	1.5/1			
VAN-SD (1 g/12 h)		56/6	299/1 = 299	299/1.5 = 199
VAN-AD (1.25 g/8 h)		96/17	621/1 = 621	621/1.5 = 414
MRSA-277	2/2			
VAN-SD (1 g/12 h)		56/6	299/2 = 149.5	299/2 = 149.5
VAN-AD (1 g/6 h)		60/20	666/2 = 333	666/2 = 333

[a]Abbreviations: VAN MIC, vancomycin MIC; MD, microdilution; AUC, area under the curve; VAN-SD, vancomycin standard doses; VAN-AD, vancomycin adjusted doses.

with vancomycin at the high doses required to achieve these targets (11, 14) limits the viability of this therapeutic approach in many clinical situations.

We aimed to compare the efficacy of vancomycin at standard doses (VAN-SD) to that of vancomycin at adjusted doses (VAN-AD) to achieve the pharmacodynamic target of an AUC/MIC of ≥400 for the treatment of experimental endocarditis caused by MRSA strains with different susceptibilities to vancomycin.

(This study was presented in part at the 52nd Interscience Conference on Antimicrobial Agents and Chemotherapy [15].)

The strains had the following microdilution vancomycin MICs/minimum bactericidal concentrations (MBCs): MRSA-196, 0.5/8 mg/liter (Etest, 0.5 mg/liter); MRSA-572, 1/1 mg/liter (Etest, 1.5 mg/liter); and MRSA-277, 2/2 mg/liter (Etest, 2 mg/liter), respectively. Their clonal complex, biofilm production, and *agr* expression studies are shown in Table S1 in the supplemental material. After randomization, the following treatment groups were obtained: (i) for all three strains, a control group of untreated animals ($n = 15$); (ii) for the MRSA-196 strain, VAN-SD ($n = 15$) and VAN-AD 1.25 g/8 h ($n = 16$); (iii) for the MRSA-572 strain, VAN-SD ($n = 15$) and VAN-AD 1.25 g/8 h ($n = 17$); and (iv) for the MRSA-277 strain, VAN-SD ($n = 15$) and VAN-AD 1 g/6 h ($n = 16$). Table 1 summarizes the pharmacodynamic profiles for the different strains and dosage regimens. The C_{min}s (minimum value ± standard deviation) obtained after 48 h of treatment with the different regimens were 3.05 ± 1.78 μg/ml for VAN-SD, 10.16 ± 5.49 μg/ml for VAN-AD 1.25 g/8 h, and 21.75 ± 6.97 μg/ml for VAN-AD 1 g/6 h.

The treatment results are shown in Table 2. As observed, all treatment regimens reduced bacterial counts in the aortic valve vegetation compared with their respective nontreatment group. However, significant differences between VAN-SD and VAN-AD regimens were not observed for any of the three strains, with similar numbers of vegetations under the two regimens becoming sterilized. Notably, the target index of AUC/MIC of ≥400 was not achieved in the VAN-AD regimen with MRSA-277, which had a vancomycin MIC of 2 mg/liter, although C_{min} in this experiment was 20 mg/liter. When comparing the groups treated with VAN-SD and VAN-AD, VAN-SD sterilized 15/45 (33.3%) vegetations, while VAN-AD sterilized 21/49 (42.8%) ($P = 0.343$). The median $\log_{10}$ CFU (interquartile range [IQR]) per gram of vegetation was 2 (0 to 6.9) for VAN-SD regimens and 2 (0 to 4.5) for VAN-AD regimens ($P = 0.384$).

This is the first study to evaluate the relationship between the achievement of a pharmacodynamic target (AUC/MIC of ≥400) and the efficacy of vancomycin at sterilizing infected valves in MRSA experimental endocarditis. Since Moise-Broder et al. observed that outcomes of patients with methicillin-resistant *S. aureus* pneumonia treated with vancomycin were better when an AUC/MIC value of 400 was achieved (12), this breakpoint has been accepted as a predictor in *S. aureus* infections by some

TABLE 2 Results after vancomycin therapy for the three strains and dosage regimens[a]

Treatment group for strain	No. of sterile vegetations/ no. of total vegetations (%)	*P* value	Median (IQR) $\log_{10}$ CFU/g vegetation	*P* value
MRSA-196				
Control	0/15		8.8 (7.9–9.5)	
VAN-SD (1 g/12 h)	6/15 (40)		2.6 (0–4.5)	
VAN-AD (1.25 g/8 h)	10/16 (62)	0.21	0 (0–3.4)	0.38
MRSA-572				
Control	0/15		10 (9.6–10)	
VAN-SD (1 g/12 h)	4/15 (27)		7 (1–7.9)	
VAN-AD (1.25 g/8 h)	3/17 (13)	0.54	5 (3–8)	0.73
MRSA-277				
Control	0/15		9 (8.6–9.3)	
VAN-SD (1 g/12 h)	5/15 (33)		2 (0–5.6)	
VAN-AD (1 g/6 h)	8/16 (50)	0.35	1 (0–2.2)	0.37
All strains				
Control	0/45		10 (9–10.1)	
VAN-SD	15/45 (33)		2 (0–6.9)	
VAN-AD	21/49 (43)	0.34	2 (0–4.5)	0.38

[a]Abbreviations: IQR, interquartile range; VAN-SD, vancomycin standard doses; VAN-AD, vancomycin adjusted doses.

authors. However, it has never been specifically evaluated for infective endocarditis. Moreover, other studies that analyzed the existence of a relationship between pharmacodynamic parameters and outcomes in patients with *S. aureus* infections either presented discordant results or did not find any association (11, 13). As a result, the recent 2015 guidelines issued by the American Heart Association (AHA) (1) and the European Society of Cardiology (ESC) (2) differed in their recommendations on the most appropriate vancomycin regimen for MRSA endocarditis. The ESC guidelines pointed out the recommendation for adjusting doses for the pharmacodynamic target of AUC/MIC of $>$400, while the AHA guidelines did not (1, 2).

In our study, we did not observe a significant benefit from increasing vancomycin doses to achieve the desired target, as the bacterial density in the vegetations was similar to that following a standard dosage therapy. Our results may be better understood by considering poor vancomycin diffusion within the vegetations (10, 12, 16). Endocardial vegetations mainly develop as biofilm-forming bacteria surrounded by a glycopeptidic layer with fibrin and platelets, which protects them from antibiotics, especially those with higher molecular weights, such as vancomycin. In addition, some of these bacteria express changes in their metabolism, allowing them to live in a nonreplicative stationary state (17, 18). Moreover, as Sakoulas et al. recently described, in endovascular staphylococcal infections, vancomycin lacks the synergy with host innate immunity observed with beta-lactams (19). Beta-lactam sensitization of host cationic peptides may be crucial in the superior activity of the vancomycin-oxacillin combination compared to vancomycin alone, as observed in our MRSA experimental endocarditis model (data not published). As a result of these shortcomings, vancomycin and other antibiotics fail to sterilize vegetations (20), and persistent infection is observed (21).

In a model similar to ours, Levine et al. treated infective endocarditis with continuous infusion of vancomycin to obtain the target of a steady-state serum level of at least 20 mg/liter. Like us, they did not observe low MICs predicting better outcomes, concluding instead that early *in vivo* results do not seem to be influenced by *in vitro* parameters (22).

In our experience, the target of AUC/MIC of $\geq$400 is not easily achievable with conventional doses, especially as microorganism MIC increases. This is especially rele-

vant provided that only 4.3% of methicillin-susceptible *S. aureus* (MSSA) and 8% of MRSA strains causing infective endocarditis at our institution during the period 1995 to 2012 (unpublished data) have a vancomycin MIC as high as 1 mg/liter. The achievement of this pharmacodynamic parameter may be limited by vancomycin's side effects, which increase at higher doses (23). An additional challenge in clinical practice is VAN MIC interpretation. This is due to the known low correlation between the two recommended methods of measurement, Etest and microdilution (24). Mention must also be made of the difficulties in measuring or targeting AUC/MICs in humans, especially in patients on hemodialysis or otherwise renally impaired, which therefore limit the use of this strategy in clinical practice. Last, another limitation is that our study focused on bacterial growth in only aortic valve vegetations and not other tissues, such as the kidney or spleen.

In summary, these findings suggest that the pharmacodynamic targets predicting outcome in MRSA bacteremia and pneumonia are less useful in MRSA experimental endocarditis, probably due to the poor diffusion of VAN within the vegetations.

Strains. Three strains of MRSA (MRSA-196, MRSA-572, and MRSA-277) isolated from patients diagnosed with infective endocarditis in our center were selected for the *in vivo* studies.

Antibiotics. VAN powder was purchased from Sigma (St. Louis, MO, USA) and was reconstituted according to the Clinical and Laboratory Standards Institute (CLSI) recommendations (25) for all experiments.

Susceptibility testing. VAN MIC and minimum bactericidal concentration (MBC) were determined by the microdilution method using cation-adjusted Mueller-Hinton broth (BMD) also according to the current CLSI recommendations (25) and by the Etest method according to the manufacturer's recommendations.

***In vivo* studies. (i) Animals.** New Zealand White rabbits (body weight, 2.2 kg) obtained from San Bernardo Farm (Pamplona, Spain) were housed in the Technological and Scientific Center (CCiT) animal facilities of the University of Barcelona as previously described (26). This research project fulfilled the requirements stipulated in Spanish Royal Decree 223/1988 on the protection of animals used in experiments. The Ethical Committee on Animal Research of the University of Barcelona approved the animal studies.

(ii) Human-like pharmacokinetics studies. In the model for human-like pharmacokinetics studies, vancomycin was administered by using a computer-controlled infusion pump system designed to reproduce human serum antibiotic levels in rabbits after an intravenous (i.v.) infusion. Pharmacokinetics studies with vancomycin have been previously described (26). To determine the animal antibiotic doses needed to simulate the human profiles of vancomycin, the AUC/MICs were calculated for the different groups using different dosing simulations. Standard (VAN-SD, 1 g/12 h) or adjusted doses to achieve the pharmacokinetic/pharmacodynamic (PK/PD) parameter AUC/MIC of $\geq$400 (VAN-AD, 1.25 g/8 h or 1 g/6 h, depending on the infective strain). In order to check VAN serum levels after 48 h of therapy, a blood sample was obtained from animals receiving three different regimens ($n = 13$ for VAN-SD 1 g/12 h, $n = 14$ for VAN-AD 1.25 g/8 h, and $n = 12$ for VAN-AD 1 g/6 h).

(iii) Endocarditis model. The experimental aortic valve endocarditis model was used as described elsewhere (26). In brief, each animal was inoculated with one of the selected MRSA strains. Sixty-four hours after the animals were infected, antibiotic therapies started and animals were treated for 48 h. The animals were treated for 48 h with an antibiotic regimen chosen by randomization. After the completion of treatment, aortic valve vegetations were obtained and qualitative cultures were performed (26).

Treatment groups. The infected rabbits were randomized to one of the different treatment arms simulating either a vancomycin human dose of 1 g/12 h (VAN-SD) or doses adjusted to achieve the pharmacodynamic target of AUC/MIC of $>$400 (VAN-AD), 1.25 g/8 h or 1 g/6 h, depending on the infecting strain.

Statistics. The median and interquartile range (IQR) of the number of $\log_{10}$ CFU per gram of vegetation were calculated for the different treatment groups and strains. The

Mann-Whitney U test was used to compare the obtained medians. The Fisher exact test was used to compare the proportion of sterile vegetations.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.02486-16.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

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