Evaluation of Once-Daily Vancomycin against Methicillin-Resistant *Staphylococcus aureus* in a Hollow-Fiber Infection Model

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For methicillin-resistant *Staphylococcus aureus* (MRSA) infections, data suggest that the clinical response is significantly better if the total vancomycin area under the concentration-time curve (AUC)/MIC ratio is $\geq 400$. While the AUC/MIC ratio is the accepted pharmacokinetic/pharmacodynamic (PK/PD) index for vancomycin, this target has been achieved using multiple daily doses. We are unaware of a systematically designed dose fractionation study to compare the bactericidal activity of once-daily administration to that of traditional twice-daily administration. A dose fractionation study was performed with vancomycin in an *in vitro* hollow-fiber infection model against an MRSA USA300 strain (MIC of 0.75 μg/ml) using an inoculum of $\sim 10^{6}$ CFU/ml. The three vancomycin regimens evaluated for 168 h were 2 g every 24 h (q24h) as a 1-h infusion, 1 g q12h as a 1-h infusion, and 2 g q24h as a continuous infusion. Free steady-state concentrations (assuming 45% binding) for a total daily AUC/MIC ratio of $\geq 400$ were simulated for all regimens. A validated liquid chromatography-tandem mass spectrometry method was used to determine vancomycin concentrations. Although once-daily and twice-daily dosage regimens exhibited total trough concentrations of $\leq 15$ μg/ml, all regimens achieved similar bactericidal activities between 24 and 168 h and suppressed the amplification of nonsusceptible subpopulations. No colonies were found on agar plates with $3 \times$ MIC for any of the treatment arms. Overall, the results suggest that once-daily vancomycin administration is feasible from a PK/PD perspective and merits further inquiry in the clinical arena.

Given the dual threat of diminishing vancomycin efficacy against methicillin-resistant *Staphylococcus aureus* (MRSA) and recent reports of rising nephrotoxicity (10, 22) (albeit potentially due to concomitant dosing increases), new vancomycin dosing strategies are urgently needed. From a pharmacodynamic viewpoint, there is an opportunity to alter standard dosage regimens of vancomycin in clinical practice to optimize outcomes and minimize toxicity. Data suggest that killing by vancomycin is concentration-dependent, and a near-maximal bactericidal effect is achieved against MRSA when the ratio of the area under the total vancomycin concentration-time curve (AUC) to the MIC exceeds 400 (22). While an AUC/MIC ratio of 400 is a well-recognized pharmacokinetic/pharmacodynamic (PK/PD) target for vancomycin, this target has been determined using multiple daily doses, which has resulted in the default stance of using multiple-daily-dosing regimens in clinical practice (4, 5, 7, 9, 12, 13, 20, 21). The possibility of once-daily administration is appealing from a PK/PD perspective, as it affords the ability to achieve more robust AUCs in a defined interval (i.e., during the first 6 to 12 h) while minimizing trough concentrations, which predicts nephrotoxicity (10). However, we are unaware of a systematically designed dose fractionation study that compared the bactericidal activity of a once-daily administration of vancomycin to that of a twice-daily administration.

Our objective was to compare the rates and extents of killing of MRSA USA300 of vancomycin regimens at 2 g/day given as a 1-h infusion every 12 h (q12h) or q24h or as a continuous infusion in a hollow-fiber infection model (HFIM). To our knowledge, this is the first dose fractionation study that compared the bactericidal activity of a once-daily administration of vancomycin to that of a twice-daily administration.

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**MATERIALS AND METHODS**

Bacterial isolates, susceptibility testing, and drug preparation. Vancomycin analytical-grade powder was purchased from Fisher Scientific (Fairlawn, NJ). A sterile stock solution of 10,000 μg/ml was prepared in sterile water, diluted and aliquoted to the appropriate concentration, and stored at $-80^\circ$C. MRSA pulsed-field gel electrophoresis type USA300 (obtained as a gift from Brian Tsuji, SUNY, Buffalo, NY) was used for susceptibility testing, time-kill studies, and the HFIM. A quality control strain (mecillin-susceptible *S. aureus* ATCC 25925) was used only for all susceptibility testing. Both of the strains were stored at $-80^\circ$C in 20% glycerol and cation-adjusted Mueller-Hinton II broth (MHB) (BBL, Sparks, MD). Prior to experimentation, fresh isolates were grown on Trypticase soy agar (TSA) plates with 5% sheep blood and incubated at 35°C for 24 h.

Susceptibility testing. Susceptibility testing was performed in triplicate using broth microdilution and agar dilution methods with MHB and Mueller-Hinton agar according to CLSI guidelines (2). Dilutions were performed by arithmetic increments of 0.25 μg/ml for the MIC range of 0.25 to 2 μg/ml and increments of 1 μg/ml for MICs of $\geq 2$ μg/ml. The minimum bactericidal concentration (MBC) was determined by the sub-culture of broth from the microdilution wells that were previously incubated for 24 h and plated onto TSA plates.
**Time-kill assay.** A 48-h time-kill experiment was conducted to evaluate the bactericidal activity of various concentrations of vancomycin against MRSA USA300. The vancomycin concentrations used in the time-kill study (0, 0.75, 2.25, 8, 16, 25, and 50 μg/ml) reflected the free-vancomycin peak and trough concentrations for the dosing regimen evaluated in the HFIM. A starting inoculum of the same density as that for the HFIM (10^5.8 CFU/ml) was used, and various concentrations of vancomycin were added to conical tubes. The time-kill experiment was performed in a water shaker bath at 35°C and at 100 rpm. Both MHB and drug were replaced after 24 h by the spinning of the bacterial suspension, removal of the supernatant, and resuspension in fresh (drug-containing) MHB in order to precisely maintain vancomycin concentrations and to maintain fresh MHB. Quantitative cultures were assessed at 0 h (predose) and 3, 6, 24, 27, and 48 h. Prior to the culturing of the bacterial samples on TSA agar, each sample was centrifuged and resuspended twice with normal saline to remove vancomycin.

**Studies of less-vancomycin-susceptible subpopulations.** The mutation frequencies of less-susceptible subpopulations at different multiples of the MIC in agar were calculated as the ratio of the number of colonies that grew on drug-containing agar divided by the number of colonies that grew on drug-free agar, as previously described by Louie et al. (11). Briefly, aliquots of the inoculum (1 to 5 ml) were plated onto agar containing 3×, 5×, 6×, and 10× the baseline vancomycin agar MIC of the organism and incubated at 35°C. After as long as 72 h of incubation, the colonies on the plates with drug-containing medium were enumerated and compared to the colony counts of the total bacterial population previously assessed at 24 h. Due to the limited bacterial growth on plates with 5×, 6×, and 10× MIC (data not shown), plates with 3× MIC were used to determine the mutational frequency and less-susceptible subpopulations in the HFIM. Determinations of mutation frequencies at 3× MIC were performed on at least four separate occasions.

**Hollow-fiber infection model.** A description of the hollow-fiber bioreactor (cellulosic cartridge; FiberCell System, Inc., Fredrick, MD) was detailed previously (24). Along with vancomycin being injected into the central compartment, a continuous flow of fresh MHB was also infused in order to represent the human simulated terminal half-life of vancomycin. In order to maintain an isovolumetric environment, MHB was removed from the central reservoir into a waste reservoir via a peristaltic pump (Masterflex; Cole-Palmer Instrument Co., Chicago, IL). In order to inoculate the HFIM, MRSA was injected and confined to the hollow-fiber cartridge (i.e., the extracapillary space of the hollow-fiber system). Broth medium including nutrients and vancomycin was exchanged by the rapid intercompartmental clearance between the central reservoir and the hollow-fiber cartridge.

**Schedule-response experiment.** A dose fractionation study was performed in duplicate with the same MRSA USA300 isolate using the HFIM in a humidified incubator at 35°C. The isolate was grown on TSA plates at 35°C. A volume of 200 μl was plated for all samples for which the lower limit of detection was 6 colonies (1.5 log₁₀ CFU/ml).

To determine susceptibility and, therefore, to confirm the development of resistance, determinations of MICs were performed in duplicate with colonies that were obtained at each time point from each experimental arm, excluding the control arm.

**LC/MS/MS assay.** The samples (vancomycin in MHB) were diluted with high-pressure liquid chromatography (HPLC) water (0.050-ml sample in 1.00 ml of a 50:50 dilution of methanol-water) and were analyzed by LC/MS/MS. The LC/MS/MS system was comprised of a Shimadzu Prominance HPLC system and a Thermo Scientific TSQ Vantage LC/MS/MS system.

Chromatographic separation was performed by using a Thermo Scientific Hypersil C18 column, a 5 μm, 50-250-mm column, and a mobile phase consisting of 85% 5 mM ammonium acetate (pH 3.5) and 15% methanol at a flow rate of 0.6 ml/min. Vancomycin concentrations were obtained by use of LC/MS/MS, monitoring the MS/MS transition from m/z 725 (doubly charged ion) to m/z 144. The analysis run time was 5.0 min. The assay was linear over a range of 0.05 to 50.0 μg/ml (r² > 0.995). The interday precision (percent coefficient of variation [%CV]) for the quality control samples (which were analyzed in triplicate at three concentrations [1.0, 5.0, and 10.0 μg/ml]) ranged from 2.68 to 3.40%; the accuracy (percent recovery) ranged from 97.5 to 101%.

**RESULTS**

**Susceptibility and mutation frequency.** The agar dilution and broth microdilution modal MICs for the MRSA USA300 strain were both 0.75 μg/ml; the MBC was 1 μg/ml. The log₁₀ mutation frequency ranged from −5.8 to −6.9 for agar plates with 3× the baseline MIC and from −7.1 to −7.7 for 5× MIC based on mutation frequencies determined on three different days. The less-susceptible subpopulations that grew in the presence of 3× MIC exhibited a higher MIC (2 to 3 μg/ml).

**Time-kill studies.** The rate of bacterial killing was 1.5 to 2 log₁₀ killing at 6 h for all tested vancomycin concentrations. Between 6 and 24 h, extensive regrowth was observed for the 0.75-μg/ml concentration. Bactericidal activity (i.e., ≥3-log kill) was observed for all concentrations within the range of 2.25 to 50 μg/ml, and the overall extent of killing and lack of regrowth up to 48 h were comparable for these concentrations (Fig. 1).

**Hollow-fiber infection model.** The observed vancomycin concentration-time profiles for the three vancomycin dosage regimens are provided in Fig. 2. The free-drug AUCs (determined by...
the linear trapezoidal rule) were well comparable between regimens, with 262.2 ± 29.5 μg·h/ml for q24h dosing, 242.1 ± 31 μg·h/ml for q12h dosing, and 244.2 ± 1.3 μg·h/ml for continuous infusion. All three vancomycin dosing regimens with a total AUC/MIC ratio of ≥400 had comparable bactericidal activities against MRSA. As shown in Fig. 3, vancomycin experienced a low rate of kill within the first 6 h (−0.91 to −1.24 log_{10} CFU/ml) and attained bactericidal activity (−3.27 to −3.57 log_{10} CFU/ml) at 24 h, which was maintained up to 168 h. Although bactericidal activity was achieved with all of the vancomycin dosing regimens in the HFIM, it is important to note that sterility was not attained. Of the colonies that did exist near or above the limit of detection for any of the dosing regimens, MICs of 0.75 to 1 g/ml did not change from the baseline. Based on the results from the drug-containing agar with vancomycin at 3× MIC, all dosing regimens prevented the breakthrough of nonsusceptible subpopulations for each of the experimental treatment arms during the 7-day HFIM experimental period. No resistant colonies were found on plates with 3× MIC for the drug treatment arms, whereas the growth control showed a number of colonies, in agreement with the mutation frequency on plates with 3× MIC.

**DISCUSSION**

After half a century of clinical use, the optimal mode of administration has not yet been elucidated for vancomycin. Although vancomycin is classified as an AUC/MIC ratio-driven antimicrobial, the data that support this were derived from multiple-daily-dosing regimens (4, 5, 7, 9, 12, 13, 20, 21). To our knowledge, this is the first contemporary dose fractionation study designed to evaluate whether killing by vancomycin is driven by the AUC/MIC ratio. Overall, when dosed to simulate a total AUC/MIC ratio of ≥400 in the HFIM, we were able to demonstrate comparable bacterial efficacies between once-daily vancomycin administration, continuous infusion, and twice-daily administration. Within each of the dosing regimens, vancomycin exhibited bactericidal activity after 24 h and maintained this activity for seven consecutive days. In addition to the sustained bactericidal effects, all three regimens suppressed the development of nonsusceptible subpopulations and were able to maintain MIC values of ≤1 μg/ml throughout the 7 days.

The exploration of dosing strategies to enhance the utility of an antibiotic agent is not a new approach (23, 25). This approach was utilized previously with daptomycin and was pivotal in the discovery that skeletal muscle toxicity issues were associated with more
frequent dosing rather than a maximum concentration (15). Similar findings were exhibited with aminoglycosides, where it was determined that more frequent dosing intervals led to higher incidences of nephrotoxicity (19). To date, we are aware of only one clinical study and one in vitro study that compared vancomycin once-daily and twice-daily dosing regimens. In the clinical study, which focused on efficacy and safety, the outcomes were comparable between once-daily and twice-daily groups; however, it should be noted that the patients studied were not severely ill (3). In the in vitro study, Houlihan et al. utilized a simulated endocarditis vegetation model at a high initial inoculum (10^9 CFU/ml) in order to investigate the use of the 2-g-per-day regimen, administered once daily, twice daily, or as a continuous infusion, for a total of 72 h (8). Similar to our study, those authors observed that trough levels did not dictate bacterial efficacy despite a bacterial inoculum as high as 10^9 CFU/ml when the total AUC/MIC ratios associated with the dosing regimens were all >400.

Our results carry important implications for clinical practice. First, they challenge the need to obtain frequent trough concentrations: bactericidal efficacy was observed despite a trough concentration that was well below the lowest recommended total trough concentration of 10 μg/ml for the total daily vancomycin dose of 2 g given as a once-daily dosing regimen. Second, these results call into question the need for trough concentrations of 15 to 20 μg/ml for all patients. Our results are similar to those of another study by our group (16), which found that regimens producing trough values of ≥15 μg/ml were not always necessary to provide a total AUC/MIC ratio of ≥400, especially if the vancomycin MIC of the MRSA isolate is ≤1 μg/ml.

A second clinical implication of our data is the convenience associated with the once-daily administration of vancomycin. Multiple daily dosing of a drug is inconvenient due to the increased nursing time and the increased risk of medication errors associated with the frequency of administration and the time intervals between doses (14). The administration of vancomycin as a once-daily drug would alleviate these inconveniences, in addition to facilitating the transition to an outpatient treatment protocol.

Finally, by minimizing the trough concentration needed to achieve the desired AUC/MIC ratio, one may be able to reduce the risk of nephrotoxicity associated with vancomycin. Since the daily AUC value is independent of the dosing frequency (16), and nephrotoxicity appears to be linked to the trough concentration (10), it may be advantageous to move from multiple daily dosing to once-daily vancomycin dosing in clinical practice. This approach needs explicit testing in randomized trials.

Overall, with a moderate inoculum, we demonstrated with an HFIM that vancomycin given as a once-daily dosing regimen exhibited bactericidal activity similar to that conferred by continuous-infusion and twice-daily dosing regimens against MRSA USA300 (MIC of 0.75 μg/ml) while maintaining the same AUC/MIC ratio of ≥400. Vancomycin was able to maintain efficacy and suppress the development of nonsusceptible subpopulations during a clinically relevant treatment duration of 7 days. Therefore, these results question the need for aggressive vancomycin trough concentrations of 15 to 20 μg/ml for all infections and suggest that the efficacy of vancomycin is not predicated on the trough concentration but rather the AUC when the MIC for the pathogen is <1 μg/ml. Finally, before these findings can be implemented in practice, clinical studies must reaffirm and strengthen the translation of the in vitro HFIM results to humans.


