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Analysis of Vancomycin Time-Kill Studies with *Staphylococcus* Species by Using a Curve Stripping Program To Describe the Relationship between Concentration and Pharmacodynamic Response

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Mono- and biexponential killing curves for vancomycin over a 2- to 50-μg/ml concentration range were generated for 11 *Staphylococcus aureus* isolates and 12 coagulase-negative *Staphylococcus* species in the logarithmic phase of growth. Nonlinear least-squares regression of the initial growth rate and disappearance were not significantly different for lower or higher concentrations of vancomycin in broth.

Interest in vancomycin has increased because of β-lactam antibiotic resistance among gram-positive cocci. Recently, the disposition of and pharmacodynamic killing by vancomycin and teicoplanin have been characterized (2–5, 13, 17). Several predictive methods that propose the modification of aminoglycoside dosing models by using desired peak concentrations of 30 to 40 μg/ml and trough concentrations of 5 to 10 μg/ml have been proposed (15, 22). Geraci and Hermans (11) recommended trough vancomycin concentrations of 8 to 10 μg/ml on the basis of an average MIC of 1 to 1.25 μg/ml, which has increased slightly since 1976. Carret et al. (7) described a unique application of a nonlinear regression program that is normally used for pharmacokinetic analysis. Killing curves generated from a range of ciprofloxacin concentrations were tested against *Escherichia coli*. The killing curves demonstrated a concentration-dependent lag phase in bacterial killing and parallel rate constants (7). The investigators (7) studied the in vitro killing of staphylococci by vancomycin in broth over a concentration range to determine whether any change in killing rate could be attributed to differences in the vancomycin concentration (1, 3, 8–10, 12, 19, 23, 24, 26, 27).

Vancomycin hydrochloride (Eli Lilly & Company) was prepared as a stock solution (10 mg/ml) in phosphate-buffered saline (pH 6.0) and was frozen at −70°C in 1.5-ml aliquots. Prepared solutions were assayed every 1 to 2 weeks by using a commercial fluorescence polarization immunoassay (TDx; Abbott Laboratories, North Chicago, Ill.) (25).

Staphylococcus aureus isolates were obtained from blood cultures (n = 8), respiratory secretions (n = 2), and a wound abscess (n = 1). Coagulase-negative *Staphylococcus* isolates were obtained from blood cultures (n = 3), abscess wound cultures (n = 4), pleural fluid culture (n = 1), urine culture (n = 1), coagulase-positive *Staphylococcus saprophyticus* (n = 1), and *Staphylococcus epidermidis* ATCC 12228 (n = 1). Standard microbiological techniques were used to differentiate *Staphylococcus aureus* from coagulase-negative species (Staphaurex latex suspension; Wellcome Diagnostics, Dartford, England).

The *Staphylococcus* species isolates were taken from fresh cultures that were 18 to 24 h old, inoculated into 1 to 2 ml of sterile Mueller-Hinton broth (Difco, Detroit, Mich.), and adjusted to a 0.5 McFarland standard. A 1:400 dilution of staphylococci in Mueller-Hinton broth provided a final inoculum of approximately 10^5 CFU/ml. At time zero, the vancomycin solution was added to the diluted bacterial suspensions in tubes at final concentrations of 50, 30, 20, 10, 8, 6, 4, and 2 μg/ml. A growth control with no added antibiotic solution was also used. All tubes were run in duplicate.

Time-kill curves were made for tubes containing bacterial suspensions and the various vancomycin concentrations incubated at 35°C. At 1, 2, 3, 4, 5, 6, 8, and 24 h postexposure, aliquots were diluted in tryptic soy broth (Difco) and plated onto blood agar plates (BBL, Cockeysville, Md.). Aliquots were made by using a clean sterile pipette tip for each tube. The number of CFU per milliliter was determined after 24 h of incubation.

Killing curves were analyzed by using PKCALC, a BASIC program containing the curve stripping program ESTRIP (Don Brown) (6), and two-compartment and three-compartment open models for oral absorption. The apparent continued growth of the bacteria in the presence of vancomycin was modeled as oral absorption, and the disappearance of organisms (in CFU per milliliter) was modeled as a mono- or biexponential decline with a possible lag time. Coefficients of determination (r^2) and the sum of the squared deviations were used for determination of goodness of fit by using established criteria (18). Exponential terms from curve stripping and the areas under the CFU per milliliter-time curve (AUCs) were grouped by vancomycin concentration. The initial growth phase, rapid organism decline, slow decline, and AUCs were grouped by high (10 to 50 mg/liter) and low (2 to 8 mg/liter) vancomycin concentrations and were analyzed by using a one-way analysis of variance (ANOVA) by seeking a difference in the means for the exponential terms (EPISTAT T.L.; Gustafson, Round Rock, Tex.). Statistical significance was defined as $P < 0.05$.

A decline in CFU per milliliter among the *S. aureus* isolates tested appeared to be biexponential in killing curves for 38 of the 88 duplicate tubes. Representative killing curves for three strains are provided in Fig. 1. Among the *S. aureus* isolates tested, the number of sigmoidal, monoexponential, and biexponential curves did not differ between curves for concentrations of ≤8 μg/ml (2, 4, 6, and 8 μg/ml) and ≥10

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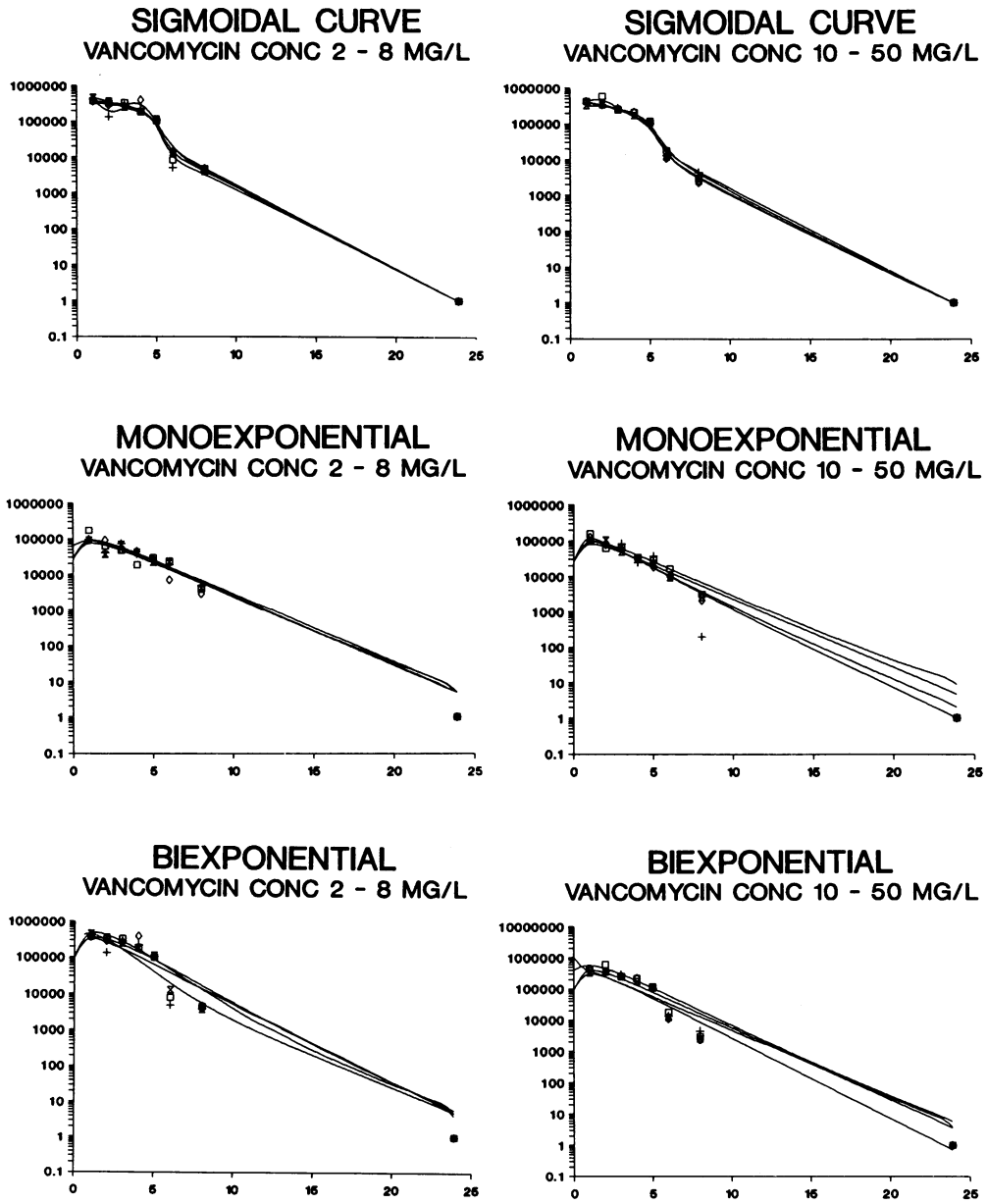

FIG. 1. Log CFU per milliliter versus time (in hours) for three pairs of representative vancomycin killing curves. Three *Staphylococcus* species isolates graphically demonstrated a sigmoidal, monoexponential, or biexponential killing curve following exposure to 2 (+), 4 (■), 6 (◆), or 8 (×) mg of vancomycin per liter versus 10 (+), 20 (■), 30 (◆), or 50 (×) mg of vancomycin per liter.

μg/ml (10, 20, 30, and 50 μg/ml). Mean monoexponential killing rates ranged from 0.4501 to 0.5080 h^{-1}. Rapid and slow mean killing rates ranged from 0.6540 to 0.8225 h^{-1} and 0.3459 to 0.4360 h^{-1}, respectively. Comparison by ANOVA of mean rapid-phase killing rates, mean slow-phase killing rates, and mean monoexponential killing rates demonstrated no real difference in the means over the range of vancomycin concentrations tested ($F = 0.6044$, 2.007, and 1.7038, respectively). ANOVA failed to demonstrate a relationship between a mean reduction in the *S. aureus* AUC and rising vancomycin concentrations. The mean initial growth rate over the vancomycin concentration range tested was not significantly different by ANOVA ($F = 1.0962$; $P > 0.05$).

Coagulase-negative *Staphylococcus* species demonstrated a biexponential decline in 32 of 96 killing curves. For coagulase-negative *Staphylococcus* species, mean monoexponential killing rates ranged from 0.5886 to 0.6077 h^{-1}. Mean biexponential killing rates ranged from 0.9064 to 1.4194 h^{-1} and 0.4318 to 0.6199 h^{-1}, respectively. For all staphylococci tested, higher or lower killing rates did not correlate with higher or lower vancomycin concentrations in broth. Comparison of initial growth in the presence of vancomycin, rapid and slow killing rates for biexponential declines in CFU per milliliter, and monoexponential killing rates by ANOVA failed to demonstrate a statistical difference ($F = 0.2275$, 0.5674, 1.3184, and 0.4120, respectively). The mean AUCs for the coagulase-negative *Staphylococcus* species also were not different.

The desired peak and trough concentrations of vancomycin have remained controversial. Many clinical laboratories report that peak concentrations of vancomycin in serum in excess of 40 μg/ml are toxic and that trough concentrations of <5 μg/ml are ineffective. Levels in excess of 40 μg/ml are potentially toxic, and the necessity of achieving peak concentrations near this range for effective killing remains to be proven. Recommendations for trough concentrations of 8 to 10 μg/ml, not 5 to 10 μg/ml, were proposed by Geraci and Hermans (11) on the basis of the MIC for *S. aureus* and the investigators' clinical experience with the drug. The model for the peak concentration range of 30 to 40 μg/ml presumes a peak concentration of five to eight times the MIC, which is analogous to aminoglycoside dosing methods. The presumption that has been made for the currently accepted therapeutic peak is that the MIC for *S. aureus* is 5 μg/ml and that vancomycin has a peak concentration-associated killing effect and a postantibiotic effect similar to those of the aminoglycosides (21). The ANOVA for the rapid and monoexponential rate constants failed to distinguish killing rates for high versus low vancomycin concentrations in broth. Failure to demonstrate a relationship between vancomycin concentration and the duration of the killing or lag phase was unexpected. This study provides some initial data which provoke serious doubts concerning the models that are used for vancomycin dosing methods and the assumptions that are presumed for therapeutic peak and trough vancomycin concentrations.

Other killing curve studies, recently published in vivo animal models, and clinical data by several investigators also challenge this hypothesis. The killing curves presented here and those recently published by Greenberg and Benes (14), who used *S. aureus* and human serum, were very similar. The serum killing curves also demonstrated a lag phase prior to a rapid killing phase. For the vancomycin concentrations in serum tested in the study described here, killing curves were again almost superimposable. Levine et al. (20) recently reviewed a series of cases of endocarditis for which vancomycin or vancomycin plus rifampin was used as empiric treatment. Persistent bacteremia was noted even with therapeutic vancomycin concentrations in serum. Kaatz et al. (16) noted that the outcome of *S. aureus* endocarditis in rabbits appeared to be related to trough rather than peak concentrations. A focus of treatment goals on peak concentrations of 30 to 40 μg/ml does not appear to ensure more rapid killing or predict better treatment responses. Additional studies should be performed to evaluate these phenomena further, to assess the impacts that these phenomena have on the goals for concentrations of vancomycin in serum commonly used in vancomycin therapeutic drug monitoring, and to adjust the doses of vancomycin used to treat patients.

REFERENCES

1. **Ackerman, B. H.** 1989. Evaluation of three methods for determining initial vancomycin doses. DICP Ann. Pharmacother. **23**:123–128.
2. **Barr, J. G., E. T. M. Smyth, and G. M. Hogg.** 1990. In vitro antimicrobial activity of imipenem in combination with vancomycin or teicoplanin against *Staphylococcus aureus* and *Staphylococcus epidermidis*. Eur. J. Clin. Microbiol. Infect. Dis. **9**:804–809.
3. **Bartoloni, A., M. G. Colao, A. Orsi, R. Dei, E. Giganti, and F. Parenti.** 1990. In-vitro activity of vancomycin, teicoplanin, daptomycin, ramoplanin, MDL 62873 and other agents against staphylococci, enterococci and *Clostridium difficile*. J. Antimicrob. Chemother. **26**:627–633.
4. **Biavasco, F., E. Giovanetti, M. P. Montanari, R. Lupidi, and P. E. Varaldo.** 1991. Development of in-vitro resistance to glycopeptide antibiotics: assessment in staphylococci of different species. J. Antimicrob. Chemother. **27**:71–79.
5. **Bingen, E., N. Lambert-Zechovsky, R. Leclercq, C. Doit, and P. Mariani-Kurkdjian.** 1990. Bactericidal activity of vancomycin, daptomycin, ampicillin and aminoglycosides against vancomycin-resistant *Enterococcus faecium*. J. Antimicrob. Chemother. **26**:619–626.
6. **Brown, R. D., and J. E. Manno.** 1978. ESTRIP, a BASIC computer program for obtaining initial polyexponential parameter estimates. J. Pharm. Sci. **67**:1687–1691.
7. **Carret, G., J. P. Flandrois, and J. R. Lobry.** 1991. Biphasic kinetics of bacterial killing by quinolones. J. Antimicrob. Chemother. **27**:319–327.
8. **Chambers, H. F., and S. Kennedy.** 1990. Effects of dosage, peak and trough concentrations in serum, protein binding, and bactericidal rate on efficacy of teicoplanin in a rabbit model of endocarditis. Antimicrob. Agents Chemother. **34**:510–514.
9. **Cooper, M. A., Y.-F. Jin, J. P. Ashby, J. M. Andres, and R. Wise.** 1990. In-vitro comparison of the postantibiotic effect of vancomycin and teicoplanin. J. Antimicrob. Chemother. **26**: 203–207.
10. **Garrison, M. W., K. Vance-Bryan, T. A. Larson, J. P. Toscano, and J. C. Rotschafter.** 1990. Assessment of effects of protein binding on daptomycin and vancomycin killing of *Staphylococcus aureus* by using an in vitro pharmacodynamic model. Antimicrob. Agents Chemother. **34**:1925–1931.
11. **Geraci, J. E., and P. E. Hermans.** 1983. Vancomycin. Mayo Clin. Proc. **58**:88–91.
12. **Gilbert, D. N., C. A. Wood, R. C. Kimbrough, and the Infectious Diseases Consortium of Oregon.** 1991. Failure of treatment with teicoplanin at 6 milligrams/kilogram/day in patients with *Staphylococcus aureus* intravascular infection. Antimicrob. Agents Chemother. **35**:79–87.
13. **Goldstein, F. W., A. Coutrot, A. Sieffer, and J. F. Acar.** 1990. Percentages and distributions of teicoplanin- and vancomycin-resistant strains among coagulase-negative staphylococci. Antimicrob. Agents Chemother. **34**:899–900.
14. **Greenberg, R. N., and C. A. Benes.** 1990. Time-kill studies with oxacillin, vancomycin, and teicoplanin versus *Staphylococcus aureus*. J. Infect. Dis. **161**:1036–1037.
15. **Hurst, A. K., M. A. Yoshinaga, G. H. Mitani, K. A. Foo, R. W. Jelliffe, and E. C. Harrison.** 1990. Application of a Bayesian method to monitor and adjust vancomycin dosage regimens. Antimicrob. Agents Chemother. **34**:1165–1171.
16. **Kaatz, G. W., S. L. Barriere, D. R. Schaberg, and R. Fekety.** 1987. The emergence of resistance to ciprofloxacin during treatment of experimental *Staphylococcus aureus* endocarditis. J. Antimicrob. Chemother. **30**:823–827.
17. **Krogstad, D. J., R. C. Moellering, and D. J. Greenblatt.** 1980. Single-dose kinetics of intravenous vancomycin. J. Clin. Pharmacol. **20**:197–201.
18. **Laskarzewski, P. M., D. L. Weiner, and L. Ott.** 1982. A simulation study of parameter estimates in one and two compartment models. J. Pharmacokinet. Biopharm. **10**:317–334.
19. **Levett, P. N.** 1991. Time-dependent killing of *Clostridium difficile* by metronidazole and vancomycin. J. Antimicrob. Chemother. **27**:55–62.
20. **Levine, D. P., B. S. Fromm, and B. R. Reddy.** 1991. Slow response to vancomycin or vancomycin plus rifampin in methicillin-resistant *Staphylococcus aureus* endocarditis. Ann. Intern. Med. **115**:674–680.
21. **Matzke, G. R., and J. M. Kovarik.** 1992. Clinical pharmacokinetics of anti-infectives, p. 34–6. *In* M. A. Koda-Kimble and L. Y. Young (ed.) Applied therapeutics: the clinical use of drugs, 5th ed. Applied Therapeutics, Vancouver, Washington.
22. **Matzke, G. R., R. W. McGory, C. E. Halstenson, and W. F. Keane.** 1984. Pharmacokinetics of vancomycin in patients with various degrees of renal function. Antimicrob. Agents Chemother. **25**:433–437.
23. **Maugein, J., J. L. Pellegrin, G. Brossard, J. Fourche, B. Leng, and J. Reiffers.** 1990. In vitro activities of vancomycin and teicoplanin against coagulase-negative staphylococci isolated

from neutropenic patients. Antimicrob. Agents Chemother. **34:**901–903.
24. **Peetermans, W. E., J. J. Hoogeterp, A.-M. Mazekamp-van Dokkum, P. van den Broek, and H. Mattie.** 1990. Antistaphylococcal activities of teicoplanin and vancomycin in vitro and in an experimental infection. Antimicrob. Agents Chemother. **34:** 1869–1874.
25. **Pfaller, M. A., D. J. Krogstad, G. G. Granich, and P. R. Murray.** 1984. Laboratory evaluation of five assay methods for vancomycin: bioassay, high pressure liquid chromatography, fluorescence polarization immunoassay, radioimmunoassay, and fluorescence immunoassay. J. Clin. Microbiol. **20:**311–316.
26. **Van der Auwera, P., M. Aoun, and F. Meunier.** 1991. Randomized study of vancomycin versus teicoplanin for the treatment of gram-positive bacterial infections in immunocompromised hosts. Antimicrob. Agents Chemother. **35:**451–457.
27. **Van der Auwera, P., G. Prinz, and G. Petrikkos.** 1991. Activity of intracellular antibiotics. Infection **19**(Suppl 4):S216–S223.