The concentration-independent effect of monoexponential and biexponential decay in vancomycin concentrations on the killing of Staphylococcus aureus under aerobic and anaerobic conditions

Alison J. Larsson*, Karla J. Walker†, Janet K. Raddatz‡ and John C. Rotschafer*‡

*Section of Clinical Pharmacy, St. Paul-Ramsey Medical Center, St. Paul, Minnesota; †Department of Pharmacy Practice, College of Pharmacy, University of Minnesota, Minneapolis, Minnesota, USA

An in-vitro pharmacodynamic system was used to generate time-kill curves to demonstrate the concentration-independent pharmacodynamics of vancomycin against Staphylococcus aureus ATCC 29213. Initial vancomycin concentrations of 5, 10, 20 and 40 mg/L were studied monoexponentially while simulating a 6 h half-life. One parallel experiment was performed in duplicate using an initial peak concentration of 40 mg/L where both a distribution α-phase half-life of 0.66 h for 1 h and an elimination β-phase half-life of 6 h for 11 h were simulated to determine if the transient distribution phase concentrations of vancomycin have any impact on bacterial killing beyond that provided by the elimination phase concentrations. Additionally, two monoexponential experiments with peak concentrations of 40 and 20 mg/L and a half-life of 6 h were repeated in an anaerobic chamber to determine if killing of S. aureus was affected.

The time to achieve a 3 log_{10} kill was calculated from the linear portion of the regression line and averaged (mean ± S.D.) 9.0 ± 1.4 h for all aerobic monoexponential experiments and was 8.4 and 8.6 h for the aerobic biexponential experiments (P > 0.05). For the anaerobic studies, the times to reach 3 log_{10} kill were significantly greater averaging 18.9 ± 1.7 h. The slopes of the bacterial kill curves were virtually identical for both monoexponential and biexponential aerobic experiments averaging –0.34 ± 0.04, yet significantly different from the anaerobic bacterial kill curve slopes of –0.16 ± 0.015 (P < 0.05). Time-kill curve analyses suggest that varying the concentration of vancomycin does not affect the rate or extent of bacterial killing aerobically or anaerobically against S. aureus and more efficient killing was achieved under aerobic conditions. The simulated distribution phase concentrations did not contribute to more effective killing of this strain of S. aureus.

Introduction

Recently, several issues have been raised regarding the use of vancomycin (Cantu, Yamanaka-Yuen & Lietman, 1994; Moellering, 1994) which undoubtedly remains the antibiotic of choice for treating infections caused by methicillin resistant staphylococci although not necessarily for those caused by methicillin susceptible strains

*Corresponding author. Tel: +1-612-221-3896; Fax: +1-612-292-4031.
A. J. Larsson et al. (Korzeniowski & Sande, 1982; Chambers, Miller & Newman, 1988; Small & Chambers, 1990; Karchmer, 1991; Levine, Fromm & Reddy, 1991). This is especially true where staphylococcal bacteraemia and endocarditis are concerned since bacteraemia persists longer and the clinical outcome is poorer in patients treated with vancomycin instead of β-lactam antibiotics (Karchmer, 1991). The glycopeptide is also being overused, not only in the United States but worldwide (Centers for Disease Control and Prevention, 1993).

Although vancomycin has been studied for over 40 years, there are few data comparing its antimicrobial activity during stationary and exponential growth or under aerobic and anaerobic conditions (Knowles et al., 1993) which are of practical concern since staphylococcal vegetations in endocarditis may contain a large number of bacteria in stationary growth and infections can occur under both aerobic and anaerobic conditions.

It has been proposed that peak serum concentrations of vancomycin should range from 30 to 40 mg/L for optimal efficacy and trough concentrations from 5 to 10 mg/L to minimise toxicity (Geraci, 1977) even though the limited data available suggest that the drug's bactericidal activity against Gram-positive bacteria is independent of its concentration (Peetermans et al., 1990; Ackerman, Vannier & Eudy, 1992). Thus, from a theoretical point of view, higher serum concentrations in patients would be unlikely to result in an increase in either the rate or extent of bacterial killing.

The purposes of this study were to determine whether vancomycin exhibited concentration-dependent or -independent activity against a strain of Staphylococcus aureus, whether the distribution or α-phase concentrations of the drug contribute to either the rate or extent of bacterial killing and whether or not anaerobic conditions adversely affect the efficiency of killing.

Materials and methods

In-vitro pharmacodynamic system

The in-vitro pharmacodynamic system used has been previously described by Zabinski et al. (1993) and consisted of a 700 mL glass vessel with inflow and outflow ports connected to a Masterflex™ peristaltic pump (Cole-Parmer Instrument Co., Chicago, IL, USA) by silicone tubing (Masterflex™ L/S thin wall tubing, Cole-Parmer, Chicago, IL, USA). The glass vessel was placed in a water bath heated by a stir-hot plate (Nuovo IT™ Barnstead/Thermolyne Corp., Dubuque, IA, USA). The temperature was maintained at 35–37°C and monitored by a thermometer in the glass vessel. A magnetic stir bar inside the glass vessel ensured constant mixing.

Sterile antibiotic-free media was pumped into the system at a predetermined rate displacing an equal amount of media containing antibiotic from the vessel into a waste reservoir. Thus, a first-order, one-compartment, pharmacokinetic process was simulated.

A half-life of 6 h was obtained by adjusting the flow rate of the peristaltic pump to 1.3 mL/min to simulate the elimination half-life of vancomycin typically encountered during therapy (Cook & Farrar, 1978; Krogstad, Moellering & Greenblatt, 1980). Peak antibiotic concentrations of 5, 10, 20 and 40 mg/L were achieved by injecting antibiotic into the glass vessel as a bolus.
Vancomycin hydrochloride (Sigma, St. Louis, MO, USA) was prepared as a stock solution of 10 mg/mL in sterile water which was stored up to 10 days at 5°C. When required for use the stock solution was diluted to achieve the initial vancomycin concentration desired.

Media

Mueller-Hinton broth (MHB; Difco, Detroit, MI, USA) was used throughout as the growth media and was supplemented with 25 mg/L calcium and 12.5 mg/L magnesium as recommended by the American National Committee for Clinical and Laboratory Standards (NCCLS, 1990) and trypticase soy blood agar (TSB; Dimed, Roseville, MN) was used to determine viable colony counts.

S. aureus ATCC 29213

S. aureus ATCC 29213 was reconstituted from stock frozen at −80°C and subcultured at least twice on TSB agar and incubated for 18–24 h at 37°C. Fifty millilitres of MHB was inoculated with two to five colonies of S. aureus and incubated at 37°C overnight. Then 15 mL were transferred to 300 mL MHB and grown for approximately 2 h to a turbidity equivalent to the number 1 McFarland’s standard. This standardised suspension was diluted 1:10 to yield approximately $1 \times 10^7$ cfu/mL as the starting inoculum of bacteria growing in the exponential phase.

Enumeration of bacteria

TSB plates were inoculated with 100 µL of serially diluted samples and incubated at 37°C for 24 h. Colony counts between 30 and 300 colonies were used to construct the time-kill curves. Inoculation of a TSB plate with 100 µL of undiluted sample resulted in a lower limit of detection of $3 \times 10^2$ cfu/mL.

Determination of the MIC and MBC

MICs and MBCs were determined aerobically and anaerobically in MHB by the standard microdilution method of the NCCLS (NCCLS, 1990) using inocula of approximately $1 \times 10^7$ and $1 \times 10^5$ cfu/mL.

Aerobic time-kill studies

Time-kill studies and growth control experiments were performed aerobically in the in-vitro pharmacodynamic system with each vancomycin concentration being tested in duplicate. All experiments were run for 12 h with 1 mL samples being drawn immediately after adding vancomycin, then 30 sec, 0.5, 1, 2, 3, 4, 6, 9 and 12 h afterwards. Each sample was serially diluted and plated onto TSB agar to determine the number of surviving bacteria. The temperature and pH were monitored throughout.

In order to simulate the natural biexponential decay of the serum concentrations of vancomycin, an initial drug concentration of 40 mg/L and a growth control were run with an α-phase half-life of 0.66 h which was achieved by adjusting the pump rate to
11.9 mL/min for the first hour. An elimination half-life of 6 h was simulated for the remaining 11 h of the experiment by lowering the pump flow rate to 1.3 mL/min. Additional samples were obtained 15 and 45 min after the addition of the drug during the α-phase experiment to estimate bacterial counts.

Anaerobic time-kill studies

Concentrations of 20 and 40 mg/L were tested anaerobically by placing the in-vitro pharmacodynamic system in an anaerobic chamber (Bactron IV; Sheldon Mfg., Cornelius, OR, USA) following the same procedures described for the aerobic experiments. An anaerobic atmosphere of 5% hydrogen, 10% carbon dioxide and 85% nitrogen was ensured by using BBL Dry Anaerobic Indicator Strips (Becton Dickinson and Co., Cockeysville, MD, USA).

Data analysis

The logarithmic decline in cfu/mL was plotted against time to establish the bacterial kill curves. Linear regression was used to determine both the time taken to achieve a 3 log₁₀ kill and the slope of the kill curves generated by each set of experiments which were then compared using analysis of variance and multiple regression. When regression analyses revealed no significant differences in either slope or time to 3 log₁₀ kill between the concentrations of vancomycin studied, an unpaired Student’s t-test was used to compare the slopes generated under aerobic and anaerobic conditions and the times to achieve 3 log₁₀ kill. A P value of <0.05 was considered statistically significant.

Results

Susceptibility to vancomycin

MICs and MBCs of vancomycin with inocula of 10⁵ and 10⁷ cfu/mL were 1.0 and 2.0 mg/L, respectively (Table I) whether or not they were determined under aerobic or anaerobic conditions.

Aerobic time-kill studies

The S. aureus grew logarithmically to a concentration of approximately 2 x 10⁶ cfu/mL and the initial α-phase appeared to have little effect on the rate and extent of growth (Figure 1). The increased flow rates required to simulate the distribution or α-phase resulted in a dilution effect during the first 2 h of the experiment.

One representative curve of each duplicate aerobic experiment is shown in Figure 1. The time to achieve a 3 log₁₀ kill was calculated from the linear portion of the kill curve.

| Table I. Vancomycin MICs and MBCs (mg/L) for S. aureus ATCC 29213 |
|-------------------------|-------------------------|
| cfu/mL | Aerobic | Anaerobic |
|      | 1 x 10⁵ | 1 x 10⁷ | 1 x 10⁴ | 1 x 10⁷ |
| MIC  | 1 | 2 | 1 | 2 |
| MBC  | 1 | 2 | 1 | 2 |
Aerobic and anaerobic activity of vancomycin

Figure 1. Time-kill curves of vancomycin vs S. aureus ATCC 29213 performed monoexponentially and biexponentially under aerobic conditions. ◇, Growth control with α-phase; ○, growth control; □, 40 mg/L; ■, 40 mg/L with α-phase; △, 10 mg/L; ●, 20 mg/L; ▲, 5 mg/L.

and was approximately 9.0 ± 1.4 h for all experiments. The slopes were similar ranging from -0.26 to -0.39. There were no statistically significant differences in the slopes of the bacterial time-kill curves (P = 0.20) nor in the times to reach a 3 log_{10} kill (P = 0.15) between the concentrations of vancomycin studied monoexponentially and biexponentially.

Table II. Summary of time-kill curve analyses under aerobic conditions

<table>
<thead>
<tr>
<th>Concentration (mg/L)</th>
<th>Time to 3 log killᵃ (h)</th>
<th>Slopeᵇ</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>8.7</td>
<td>-0.35</td>
</tr>
<tr>
<td>5</td>
<td>7.7</td>
<td>-0.39</td>
</tr>
<tr>
<td>10</td>
<td>8.1</td>
<td>-0.37</td>
</tr>
<tr>
<td>10</td>
<td>7.7</td>
<td>-0.39</td>
</tr>
<tr>
<td>20</td>
<td>11.7</td>
<td>-0.26</td>
</tr>
<tr>
<td>20</td>
<td>9.7</td>
<td>-0.31</td>
</tr>
<tr>
<td>40</td>
<td>10.2</td>
<td>-0.29</td>
</tr>
<tr>
<td>40</td>
<td>8.2</td>
<td>-0.37</td>
</tr>
<tr>
<td>40 (α-phase)</td>
<td>8.6</td>
<td>-0.35</td>
</tr>
<tr>
<td>40 (α-phase)</td>
<td>8.4</td>
<td>-0.36</td>
</tr>
</tbody>
</table>

ᵃP = 0.15;ᵇP = 0.20.
Table III. Data comparison of anaerobic vs aerobic experiments

<table>
<thead>
<tr>
<th>Concentration (mg/L)</th>
<th>Time to 3 log kill* (h)</th>
<th>Slope* anaerobic/aerobic</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>16.5/9.7</td>
<td>-0.18/-0.31</td>
</tr>
<tr>
<td>20</td>
<td>19.2/11.7</td>
<td>-0.16/-0.26</td>
</tr>
<tr>
<td>40</td>
<td>19.5/8.2</td>
<td>-0.15/-0.37</td>
</tr>
<tr>
<td>40</td>
<td>20.4/10.2</td>
<td>-0.15/-0.29</td>
</tr>
</tbody>
</table>

*P = 0.0002; †P = 0.0009.

Anaerobic time-kill studies

A 3 log₁₀ kill was not achieved during the 12 h of the anaerobic experiments. The times to achieve a 3 log₁₀ kill at a concentration of 20 mg/L anaerobically were 16.5 and 19.2 h compared to 9.7 and 11.7 h aerobically (Table III). The calculated times to achieve a 3 log₁₀ kill at a concentration of 40 mg/L anaerobically were 16.6 and 19.2 h compared to 8.2 and 10.2 h aerobically. A statistically significant difference was found in the times to achieve a 3 log₁₀ kill between the aerobic and anaerobic experiments (P = 0.0002).

The slopes from the experiments run at 20 mg/L anaerobically were −0.18 and −0.16 compared with −0.31 and −0.26 obtained from those performed aerobically. The corresponding slopes from the experiments run at 40 mg/L anaerobically were both −0.15 compared to −0.37 and −0.29 aerobically. The difference in the slopes of the bacterial time kill curve generated under aerobic and anaerobic conditions was statistically significant (P = 0.0009).

Figure 2. Time-kill curves for *S. aureus* ATCC 29213 performed under aerobic and anaerobic conditions.

- △, Anaerobic growth control; □, 20 mg/L anaerobic; ■, 40 mg/L anaerobic; ○, 20 mg/L aerobic; ●, 40 mg/L aerobic.
Aerobic and anaerobic activity of vancomycin

Discussion

In this study, the concentration of vancomycin was constantly above the MIC for the \textit{S. aureus} during the 12 h that each experiment ran, demonstrating that the glycopeptide exerts its activity independent of the concentration. Varying the concentration did not significantly affect the slope of the linear portion of the kill curve of the \textit{S. aureus} nor the time to achieve a 3 log\textsubscript{10} kill. Bacterial counts declined slightly faster during the initial distribution or \(t\)-phase as a result of the dilution created by the rapid pump rate required to simulate the distribution half-life of 40 min. The rapidly changing concentrations of vancomycin simulated in the distribution phase did not influence the outcome of the experiment when this effect was taken into account. Both the rate and extent of bacterial kill were not different when compared to monoexponential experiments.

Contrary to the findings of Knowles \textit{et al.} (1993), vancomycin appeared to be less effective under anaerobic conditions but the rate and extent of bacterial kill was still independent of concentration although the drug was less efficient in killing \textit{S. aureus} than was found under aerobic conditions. As these experiments involved a single 12 h exposure to vancomycin \textit{in vitro} under anaerobic conditions, it is difficult to translate them to clinical circumstances in which vancomycin is given in multiple doses for several days.

Vancomycin has traditionally been given in sufficient doses to achieve peak concentrations (bound and unbound) of 30–40 mg/L and trough concentrations of 5–10 mg/L (Geraci, 1977; Rotschafer, 1986; Fitzsimmons, Postelnick & Tortorice, 1988). While identifying the optimal pharmacodynamic measure of outcome remains controversial, the time that unbound vancomycin concentrations remain above the bacterial MIC would seem to represent a reasonable predictor of outcome given the data currently available.

Vancomycin's large volume of distribution (0.7 L/kg) combined with a modest degree of protein binding (30–50\%) would likely produce effective drug concentrations at the site of infection provided a standard serum-concentration-time curve is established (Rotschafer, 1986; Rodvold \textit{et al.}, 1988; Zokufa \textit{et al.}, 1989; Garrison \textit{et al.}, 1990; Albrecht \textit{et al.}, 1991; Sun, Maderazo & Krusell, 1993). The concentrations of vancomycin modelled in our experiments ranged from 5 to 40 mg/L. We have previously demonstrated good correlation between the albumin bound and free fraction vancomycin in pharmacodynamic studies indicating that the drug is not bound to constituents in the media (Garrison \textit{et al.}, 1990). Assuming that approximately 40\% of vancomycin is bound to protein in patients, the drug levels simulated in these experiments would potentially represent total (bound and unbound) serum concentrations of 12.5–100 mg/L. Thus, there would appear to be little, if any, rationale for increasing the serum concentrations beyond the currently defined therapeutic range. Given these in-vitro data, vancomycin concentrations within the currently defined therapeutic range are likely to prove bactericidal when treating most infections although larger doses may be required when treating meningitis to improve penetration into the central nervous system and for patients with burns who may be hyperdynamic. Consequently, attempts to exceed these doses would seem futile as the resulting serum concentrations are unlikely to result in a more rapid rate or extent of kill.

The analyses of the time-kill curves generated in this study indicate that vancomycin acts independently of concentration and that the drug's concentrations during the
distribution phase do not provide any additional bactericidal activity to that attained by concentrations during the elimination phase. Therefore, when considering an appropriate dosage regimen of vancomycin, the unbound concentration of drug should probably be maintained above the MIC for most, if not all, of the dosage interval to achieve optimal activity.

References


Aerobic and anaerobic activity of vancomycin


(Received 2 August 1995; accepted 25 March 1996)