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Effects of Dosage, Peak and Trough Concentrations in Serum, Protein Binding, and Bactericidal Rate on Efficacy of Teicoplanin in a Rabbit Model of Endocarditis

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The effect of dosage and the relative importance of peak and trough concentrations in serum for efficacy of teicoplanin were examined in a rabbit model of aortic valve endocarditis. Concentrations of teicoplanin in serum exceeded the MIC by several hundredfold, yet teicoplanin was less rapidly bactericidal than penicillin both in vitro and for endocarditis caused by a strain of *Streptococcus sanguis*. Because teicoplanin was 90% protein bound in rabbit serum, low free-drug concentrations probably resulted in less activity in vivo than in vitro. Because teicoplanin has a relatively low bactericidal rate and a high degree of protein binding, a sustained concentration in serum several times greater than the MIC may be important for efficacy in vivo. An intravenous regimen with relatively high peak concentrations in serum was less effective than an intramuscular regimen for endocarditis caused by a strain of *Staphylococcus aureus*, indicating that high peaks are unlikely to be an important determinant of efficacy. The therapeutically more relevant concentration in serum may be the trough.

Teicoplanin is a parenteral glycopeptide antibiotic with an antibacterial spectrum like that of vancomycin (1, 9). Unlike vancomycin, teicoplanin is well tolerated after intramuscular administration. Its half-life in humans is 32 h or longer (14). Because of its long half-life, once-daily dosing may be possible for treatment of serious infections.

In clinical trials employing relatively low doses of 200 to 400 mg once daily, however, teicoplanin was less effective for treatment of serious staphylococcal infections than was a standard regimen (3). Treatment failures have occurred in open trials (2, 10), and inability to achieve adequate concentrations in serum with recommended dosages has been implicated as a cause of failure (11). Recently, a multicenter study comparing teicoplanin with vancomycin for treatment of staphylococcal bacteremia and endocarditis was halted when interim analysis suggested that teicoplanin was less effective than vancomycin (D. N. Gilbert, C. A. Wood, R. Bracis, R. C. Kimbrough, and the Infectious Diseases Consortium of Oregon, Program Abstr. 28th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 935, 1988).

In an effort to understand factors that might contribute to therapeutic failure, the experiments described below were conducted to examine the relationship between concentrations of teicoplanin in serum and efficacy of the drug in a rabbit model of aortic valve endocarditis.

MATERIALS AND METHODS

Bacterial strains. *Streptococcus sanguis* Poise and methicillin-resistant *Staphylococcus aureus* 67-O (4) were used to establish endocarditis.

In vitro susceptibility. MICs were determined by macrodilution (12) in Todd-Hewitt broth for the streptococcal strain and in Mueller-Hinton broth for the staphylococcal strain with inocula of approximately 2×10^5 CFU/ml. MICs were read after the strains were incubated at 37°C for 24 h. MBCs were determined by quantitative culture of broth at each concentration that showed no visible growth. The MBC was defined as the concentration yielding a 99.9% reduction in the number of viable counts.

Time-kill studies were performed at 37°C in 10 ml of Trypticase (BBL Microbiology Systems) soy broth (TSB) and in TSB plus 50% pooled rabbit serum, with inocula of 3×10^5 CFU/ml. An antibiotic concentration four times the MIC was used. Samples (100 μl) were taken at 0, 4, and 24 h and quantitatively subcultured onto blood agar. Colonies were counted after a 24-h incubation at 37°C.

Protein binding. Binding of teicoplanin to serum proteins was determined by ultrafiltration centrifugation (6). Pooled rabbit serum was spiked with either 5 or 40 μg of teicoplanin per ml or with either 1 or 10 μg of penicillin G per ml. The samples were centrifuged in an ultrafiltration membrane centrifugation device with a 10,000-dalton molecular mass cutoff (Centricon Microconcentrator; Amicon Corp., Danvers, Mass.) at $5,000 \times g$, for 25 min at 4°C. Concentrations in filtrate and spiked serum were measured by agar diffusion assay (described below), and standard curves were prepared by using filtrate or pooled rabbit serum, respectively. The percentage of protein binding was calculated as [1 − (filtrate drug concentration/serum drug concentration)] × 100%.

Assay of drug concentrations. Teicoplanin and penicillin G concentrations in serum were measured in triplicate by agar diffusion assay (15). *Bacillus subtilis* ATCC 6633 was used as the test organism. For teicoplanin, Mueller-Hinton II agar (BBL) supplemented with 3% NaCl, 0.8% $CaCl_2$, and 0.1% citric acid, pH 5.1, was used. For penicillin, Penassay medium (Difco Laboratories) was used.

The coefficient of variation for the teicoplanin assay was 16%. The lower limits of detection were 0.6 μg/ml for serum samples and approximately 2 μg/ml for vegetations; the upper limits were 50 μg/ml. The standard curve was linear ($r^2 \geq 0.99$) over this range.

Concentrations of teicoplanin in vegetations were assayed by the method used for serum. Vegetations were rinsed with 0.9% NaCl to minimize drug carry-over from blood, diluted

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in pooled rabbit serum (ratio of 0.1 g of vegetation per 0.2 ml of serum), and then homogenized. In preliminary studies, two standard curves were determined: one with teicoplanin added to pooled rabbit serum and the other with teicoplanin added to homogenized vegetations obtained from infected, untreated rabbits. Once the two methods were shown to be the same, the standard curve was determined with pooled serum as the diluent.

Streptococcal endocarditis model. New Zealand White rabbits weighing approximately 2 kg were used. To establish endocarditis, a catheter was positioned across the aortic valve and secured in place for the duration of the experiment (13). Twenty-four hours later, 1 ml of 0.9% NaCl containing 10^7 to 10^8 CFU of *S. sanguis* Poise per ml was injected intravenously. Treatment began 48 h after infection. Treatment regimens were 600,000 U of procaine penicillin every 12 h intramuscularly for each rabbit or 4.5, 9, or 18 mg of teicoplanin per kg of body weight every 12 h intramuscularly. The first dose of teicoplanin was doubled for loading. Duration of therapy was either 2 or 5 days. Blood samples for assay of peak concentrations in serum were obtained 1 h after the third dose.

Untreated rabbits were sacrificed at the beginning of therapy. Rabbits treated for 2 days were sacrificed 12 h after the last dose. Blood for the determination of trough concentrations in serum was obtained at this time. Rabbits treated for 5 days were sacrificed 6 days after the last dose. Vegetations were aseptically removed, weighed, and homogenized in 0.5 ml of 0.9% saline.

The numbers of organisms in vegetations were determined by quantitative culture onto blood agar plates, which were incubated for 48 h at 37°C. The result was expressed as the vegetation titer, defined as $\log_{10}$ CFU per gram of vegetation. Because all undiluted vegetation homogenate was cultured, as few as 5 to 10 CFU/g of vegetation could be detected. Sterile vegetations were assigned a value of 0 $\log_{10}$ CFU/g (corresponding to 1 CFU/g).

Staphylococcal endocarditis model. *S. aureus* 67-O was used to establish endocarditis. The inoculum was 1 ml of 0.9% NaCl containing 10^6 CFU/ml. Therapy began 24 h after infection and continued for 4 days.

The purpose of these studies was to determine whether higher peaks or higher troughs were more important therapeutically. Therefore, the intravenous route of administration was compared with the intramuscular route of administration for the same dosage of teicoplanin, which was 36 mg/kg as a loading dose and 18 mg/kg every 12 h.

Separate studies were conducted to characterize serum teicoplanin concentrations produced by each route of administration. Blood samples were obtained from infected rabbits at several time points after a single 36-mg/kg dose, given either intravenously or intramuscularly. Rabbits for these studies were sacrificed after the 24- to 26-h blood sampling period and were not included in the treatment experiments.

For the 4-day treatment experiments, untreated rabbits were sacrificed at the beginning of therapy. Treated rabbits were sacrificed 12 h after the eighth dose. Vegetations were removed and processed as described above.

Statistical analysis. Parametric data were expressed as means ± standard deviations. Student's unpaired *t* test was used when two groups were compared, and analysis of variance and the Newman-Kuels test were used when more than two groups were compared. The two-tailed Fisher exact test was used to compare nonparametric data. Statistical significance was defined as $P < 0.05$.

RESULTS

Susceptibility and protein binding studies. MICs for the *S. sanguis* strain were 0.06 μg of teicoplanin per ml and 1 μg of penicillin per ml. The MBC equalled the MIC for each drug. MICs in broth containing 50% pooled rabbit serum were 0.5 μg of teicoplanin per ml and 1 μg of penicillin per ml.

For the *S. aureus* strain, the teicoplanin MIC was 1 μg/ml and the MBC was 2 μg/ml. The MIC was 4 μg/ml in broth containing 50% serum.

In time-kill studies of the *S. sanguis* strain in TSB at concentrations ranging between 2 and 16 times the MIC, the rate of kill for penicillin was constant and independent of concentration. The rate for teicoplanin was also constant over this range above the MIC.

Penicillin was more rapidly bactericidal than teicoplanin. Studies at four times the MIC, both in TSB and in TSB plus 50% serum, are shown in Fig. 1. Penicillin produced an approximately 3-$\log_{10}$ decrease in CFU by 4 h in TSB and in TSB plus serum. Teicoplanin in TSB produced less than a 1-$\log_{10}$ decrease by 4 h and a 3-$\log_{10}$ decrease by 24 h. In the presence of 50% serum, teicoplanin was bacteriostatic, with less than a 1-$\log_{10}$ decrease even after 24 h. This suggested that significant amounts of teicoplanin might be bound to serum proteins.

Protein-binding studies showed that teicoplanin was highly protein bound. Approximately 90% of the teicoplanin versus approximately 68% of penicillin was bound to serum proteins (Table 1).

Streptococcal endocarditis. In vivo, the mean concentrations of penicillin in serum that were achieved exceeded the MIC for the *S. sanguis* strain by 6- to 10-fold (Table 2). The mean concentrations of teicoplanin in serum were 200 to 600 times higher than the MIC 1 h after a dose and 60 to 90 times the MIC at trough.

Despite the relatively unfavorable ratio of the penicillin MIC to the mean concentration in serum compared with this ratio for teicoplanin, rabbits treated with penicillin had significantly fewer residual organisms in vegetations after 2 days than did rabbits treated with the two lower doses of teicoplanin ($P < 0.025$ and $P < 0.005$) (Table 3). At the highest doses, mean vegetation titers for teicoplanin were still higher than for penicillin, but the difference was not statistically significant. After 5 days of therapy, penicillin and teicoplanin were similarly effective (Table 3).

Results from the 2-day treatment experiments suggested that efficacy of teicoplanin was dose dependent even at concentrations in serum greatly in excess of the MIC. A decrease of 1 $\log_{10}$ CFU/g in mean vegetation titers occurred with each doubling of the teicoplanin dose. A follow-up series of experiments, which were controlled for the potential problem of carryover of teicoplanin in vegetations (and, therefore, artifactually lower titers in rabbits given the high-dose regimen), was conducted to confirm this impression.

In these experiments, rabbits again were treated for 2 days, but this time, rabbits were sacrificed 5 days after the last dose (i.e., 15 beta-elimination half-lives of teicoplanin), instead of 12 h after the last dose. Regimens used were either 9 mg of teicoplanin per kg as a loading dose and then 4.5 mg/kg every 12 h intramuscularly or 36 mg/kg as a loading dose and then 18 mg/kg every 12 h intramuscularly. At the lower dosage, 6 of 13 rabbits had sterile vegetations and mean vegetation titers of 2.4 ± 3.0; at the higher dosage, 13 of 14 rabbits had sterile vegetations and titers were 0.5 ± 1.8 ($P = 0.013$ by the Fisher exact test).

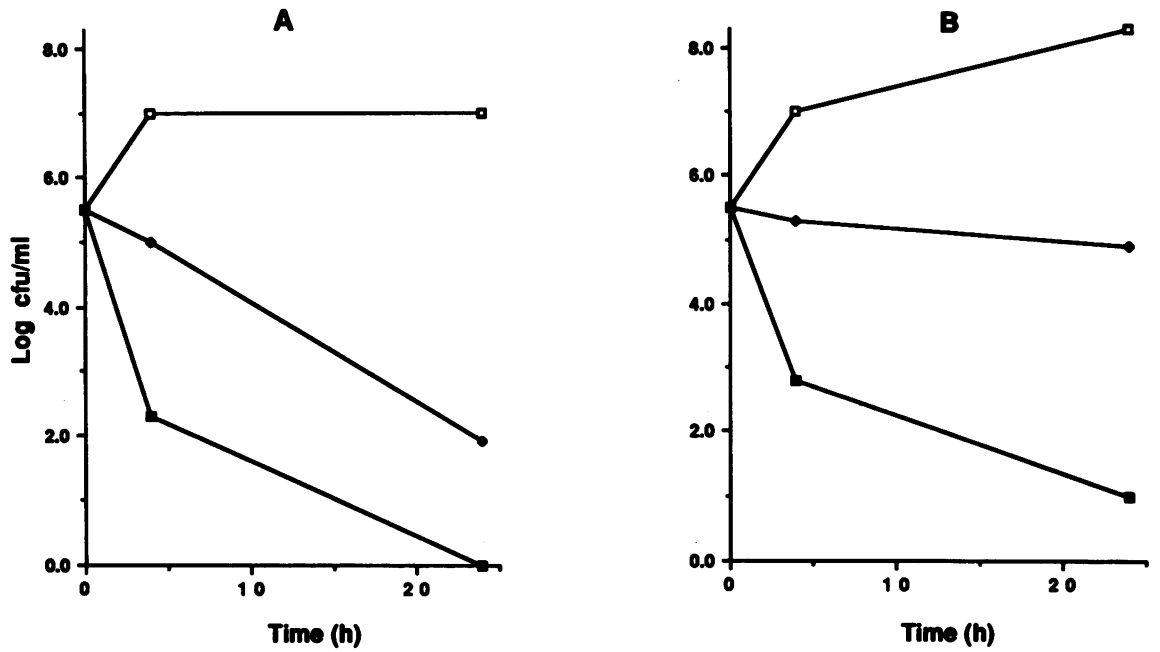

FIG. 1. Time-kill studies for teicoplanin and penicillin against *S. sanguis* Poise in TSB (A) or in TSB plus 50% pooled rabbit serum (B). Symbols: □, untreated control culture; ◆, teicoplanin; ■, penicillin.

Staphylococcal endocarditis. To investigate further this relationship between concentration of teicoplanin in serum and efficacy, the relative importance of peak and trough concentrations was examined. Would a regimen producing very high peak concentrations be more effective or less effective than one of the same total daily dosage producing lower peaks but higher troughs?

To answer this question, an intravenous dosing regimen, with higher peaks, was compared with an intramuscular regimen, with higher troughs (Fig. 2), for endocarditis caused by a strain of *S. aureus*. The staphylococcal strain was used instead of the streptococcal strain because the staphylococcal strain has a higher MIC and because it is more difficult to eradicate. If a difference in efficacy existed, it probably would be more readily detectable in an infection that was less responsive to treatment.

Concentrations in serum achieved in infected rabbits 30 min after a single 36-mg/kg dose of teicoplanin were 276 ± 30 μg/ml (n = 3) (intravenous dose) and 30 ± 12 μg/ml (n = 3) (intramuscular dose); after 2 h, concentrations were 168 ± 28 μg/ml (n = 3) (intravenous dose) and 68 ± 14 μg/ml (n = 3) (intramuscular dose) (Fig. 2). Thus, peak concentrations produced by intravenous injection exceeded those produced by intramuscular injection by 2.5 to 9 times.

At 12 h and later after dosing, concentrations in serum produced by the intramuscular regimen were significantly higher than those produced by the intravenous regimen (P = 0.012). At 12 h after dosing, the concentrations in serum were 26 ± 11 μg/ml (n = 7) with intravenous injection and 34 ± 10 μg/ml (n = 6) with intramuscular injection; thereafter, concentrations in serum averaged 30% higher with the intramuscular regimen. Both beta-elimination phase half-lives were approximately 8 h. The relative bioavailability (calculated as the ratio of the areas under the concentration-time curves extrapolated to infinity) of the intramuscular route was 80% of that of the intravenous route, which may account for the somewhat-lower-than-expected mean trough concentration with intramuscular dosing.

Despite much higher peak concentrations in rabbits given the intravenous regimen, rabbits given intramuscular teicoplanin had lower mean vegetation titers (P = 0.014) (Table 4). The mean concentrations of teicoplanin in serum at the time of sacrifice were 11 ± 9 μg/ml (n = 7) for rabbits treated intravenously versus 16 ± 11 μg/ml (n = 7) for those treated intramuscularly. Teicoplanin concentrations in vegetations of rabbits treated intravenously (two of which had undetectable teicoplanin concentrations) were 8 ± 7 μg/g (n = 5; range, <2 to 15 μg/g) versus 17 ± 11 μg/ml (n = 3; range, 7 to 28 μg/g) for vegetations of rabbits treated intramuscularly.

TABLE 1. Protein binding of teicoplanin and penicillin in spiked pooled rabbit serum

Drug	Concn in serum (μg/ml)	Free drug (μg/ml)	Protein binding (%)
Teicoplanin	5	0.4	93
	40	5.2	87
Penicillin	1	0.23	77
	10	4.1	59

TABLE 2. Mean concentrations in serum in peak and trough samples

Drug and dose (q12h)[a]	Mean concn (μg/ml) in serum ± SD (n)	
	Peak (1 h)	Trough (12 h)
Penicillin, 600,000 U	12 ± 4 (6)	5 ± 2 (7)
Teicoplanin		
4.5 mg/kg	13 ± 3 (6)	4 ± 3 (7)
9 mg/kg	21 ± 7 (6)	4 ± 2 (8)
18 mg/kg	36 ± 16 (5)	6 ± 4 (8)

[a] q12h, Every 12 h.

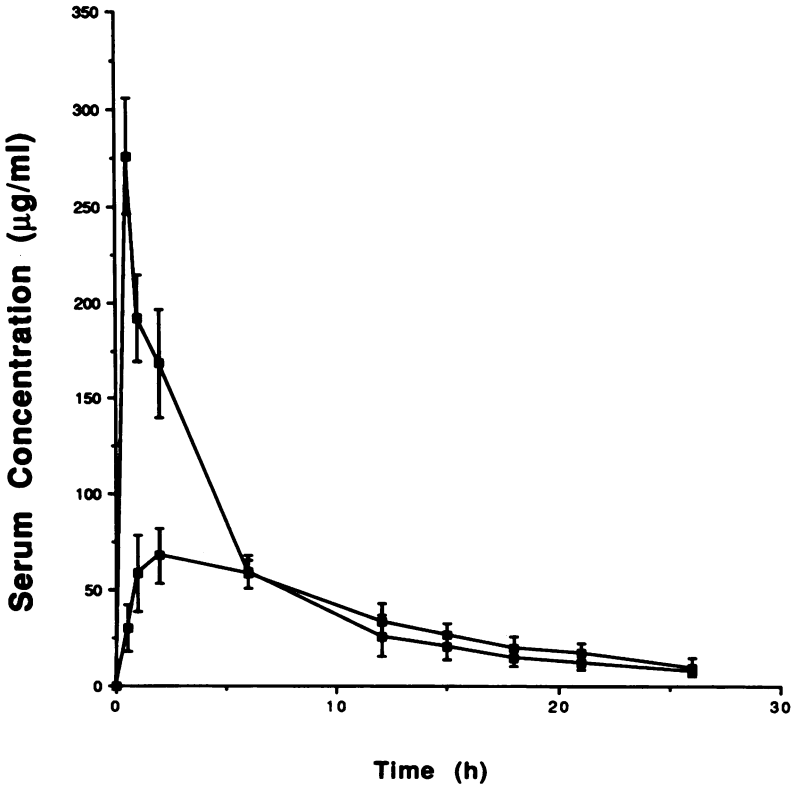

FIG. 2. Mean teicoplanin concentrations in serum in rabbits with *S. aureus* endocarditis after a single 36-mg/kg dose administered either intravenously (□) or intramuscularly (■). Error bars show standard deviations.

Differences in concentrations between the two regimens did not achieve statistical significance.

DISCUSSION

After 2 days of therapy, penicillin was more effective than teicoplanin, especially at lower doses, in reducing vegetation titers for *S. sanguis* endocarditis in rabbits. The efficacy of teicoplanin was dose dependent. A regimen of 18 mg/kg sterilized 93% of vegetations compared with 48% with a regimen of 4.5 mg/kg.

Interestingly, with 2 days of treatment, more vegetations were sterile and the titers were lower in rabbits sacrificed 5 days after the last dose than in rabbits sacrificed 12 h after the last dose. Because the experiments were performed sequentially and not simultaneously, this difference may be due simply to day-to-day variability of the model. Alternatively, low residual concentrations of teicoplanin, perhaps slowly released from protein binding sites within the vegetation, may be sufficient to continue killing already injured organisms.

Two properties of teicoplanin that could account for its being slightly less efficacious than penicillin are a lower rate of kill and a high degree of protein binding. Teicoplanin was less rapidly bactericidal than penicillin in time-kill studies. The rate at which bacteria are killed is an important component of bactericidal activity (8).

Teicoplanin was more highly protein bound than penicillin, i.e., 90% compared with 68%. A high degree of protein binding with low free-drug concentrations of drug could contribute to less-than-expected efficacy in vivo (5). In vitro MICs in 50% serum were higher for both the streptococcal and staphylococcal strains. In time-kill studies, teicoplanin

TABLE 3. Mean vegetation titers and rates of positive cultures in rabbits infected with *S. sanguis* Poise and treated with a 2- or 5-day regimen

Therapy (q12h)[a]	Mean titer in vegetation ± SD (log_{10} CFU/g) (no. positive cultures/no. inoculated)	
	2-day regimen	5-day regimen
None	8.8 ± 0.5 (11/11)	ND[b]
Penicillin, 600,000 U	1.4 ± 1.2 (11/16)	0 ± 0 (0/6)
Teicoplanin		
4.5 mg/kg	5.3 ± 3.0 (4/5)	1.0 ± 2.5 (2/12)
9 mg/kg	4.3 ± 2.6 (7/9)	1.2 ± 2.9 (2/13)
18 mg/kg	3.1 ± 3.3 (4/8)	ND

[a] q12h, Every 12 h.
[b] ND, not done.

TABLE 4. Mean vegetation titers of rabbits infected with *S. aureus* 67-O and treated with intravenous or intramuscular teicoplanin[a]

Treatment[b]	Mean vegetation titer ± SD (log_{10} CFU/g)	No. of infected rabbits/ total no. of rabbits
None	8.9 ± 0.9	5/5
i.v.	3.7 ± 2.6	9/12
i.m.	1.2 ± 1.6	4/9

[a] A 36-mg/kg loading dose of teicoplanin and 18-mg/kg doses every 12 h were administered.
[b] i.v., Intravenous; i.m., intramuscular.

at four times the MIC was bacteriostatic against the streptococcal strain, whereas penicillin was bactericidal.

Protein binding also may be a component of the reported poor penetration of teicoplanin into vegetations. Cremieux and colleagues (7) have shown that teicoplanin concentrates at the periphery of the vegetation and diffuses poorly into the center. If teicoplanin binds tightly to proteins in the infected vegetation, this could impede penetration to the site of infection and further impair the activity of the drug.

We hypothesized that a barrier to the penetration of teicoplanin might be overcome by dosage regimens producing very high peak concentrations in serum. A recent clinical trial of teicoplanin used to treat endocarditis (11) suggested that high peak concentrations in serum are important. Surprisingly, an intramuscular regimen, which produced substantially lower peak concentrations, was more effective than an intravenous regimen. The intramuscular regimen was more effective in each of the three individual experiments that produced this overall result, making chance or variability in the model an unlikely explanation for this observation.

If the peak concentration is not a critical determinant of efficacy, then what might the critical determinant be? Bioavailability and area under the concentration-time curve were slightly greater for the intravenous regimen. This cannot account for the results.

A possible explanation for the better result with the intramuscular regimen is that higher troughs, longer periods of time above a critical concentration, and perhaps more time for penetration of teicoplanin into the vegetation were produced by the intramuscular regimen. In support of this, both trough and vegetation concentrations were higher with the intramuscular regimen. Corrected for 90% protein binding, the calculated mean of free-drug concentrations in vegetations was below the MIC for the staphylococcal strain with the intravenous regimen and above the MIC with the intramuscular regimen. These differences, however, did not reach statistical significance, possibly because of the small number of samples and the relatively large individual variability of trough concentrations among rabbits.

These studies suggest that teicoplanin may be less rapidly bactericidal than penicillin both in vitro and in vivo. However, differences in vivo were statistically significant only at the two lower doses of teicoplanin after 2 days of therapy and were not present after 5 days. The clinical significance of this observation is unclear, particularly when a prolonged course of therapy is being prescribed, as in treatment of endocarditis. On the other hand, rapid sterilization of the vegetation and bloodstream may be important in preventing valvular damage and metastatic infectious complications that could occur with a more prolonged period of active infection.

These studies also suggest that protein binding may reduce the activity of teicoplanin in vivo compared with in vitro. Paradoxically, a regimen providing very high peak concentrations of teicoplanin was less effective than one producing much lower peaks and slightly higher troughs. Thus, it may be advisable to determine teicoplanin dosages on the basis of trough, not peak, concentrations when treating serious infections such as endocarditis. Assuming 90% protein binding, the trough concentration in serum may have to be as much as 10 times the MIC.

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