ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 1994, p. 487–493
0066-4804/94/$04.00+0

Vol. 38, No. 3

Role of Tolerance in Treatment and Prophylaxis of Experimental *Staphylococcus aureus* Endocarditis with Vancomycin, Teicoplanin, and Daptomycin

G. P. VOORN,[1]† J. KUYVENHOVEN,[1] W. H. F. GOESSENS,[2] W. C. SCHMAL-BAUER,[1] P. H. M. BROEDERS,[1] J. THOMPSON,[1]* AND M. F. MICHEL[2]

Department of Infectious Diseases, University Hospital, Leiden,[1] and Department of Clinical Microbiology and Antimicrobial Therapy, Erasmus University, Rotterdam,[2] The Netherlands

Received 1 June 1993/Returned for modification 30 September 1993/Accepted 8 December 1993

The role of *Staphylococcus aureus* tolerance in the treatment and prophylaxis of endocarditis in rats was investigated. The efficacies of vancomycin, teicoplanin, and daptomycin, alone and in combination with rifampin, were compared in rats with endocarditis infected with a tolerant strain of *S. aureus* and in rats with endocarditis infected with its nontolerant variant. In vitro the cloxacillin-tolerant strain was also tolerant to vancomycin and teicoplanin, but not to daptomycin. However, tolerance to these antibiotics did not influence the results of treatment of experimental *S. aureus* endocarditis. There was no difference in the bacterial densities in the vegetations of rats infected with either the tolerant or the nontolerant strain after 5 days of treatment with any of the antibiotic regimens. Of all antibiotics, daptomycin was the most effective in reducing bacterial numbers in vegetations. Combination of rifampin with vancomycin or teicoplanin improved the results of treatment for the tolerant as well as the nontolerant strains. Daptomycin was as effective alone as in combination with rifampin. In contrast, tolerance influenced the prophylactic effects of vancomycin and teicoplanin. The proportion of rats with sterile vegetations after prophylaxis with vancomycin or teicoplanin at a low dose was lower for those infected with the tolerant strain than for those infected with the nontolerant strain. A low dose of daptomycin was equally effective against the tolerant and the nontolerant strains. However, higher doses of all three antibiotics afforded almost full protection against both strains.

Tolerance, as described by Tomasz et al. (32), is a type of resistance in which antibiotics at concentrations that are bactericidal for nontolerant strains inhibit bacterial growth without killing the microorganisms. This phenomenon has been described for many bacterial species, including *Staphylococcus aureus*. The tolerance phenomenon may have clinical relevance for infections such as bacterial endocarditis, in which killing of the bacteria depends mainly on antibiotic action. However, conflicting results about the significance of tolerance in humans have been reported (14). In animals with experimental streptococcal endocarditis, tolerance seems to be important for both treatment and prophylaxis (4, 16, 20, 25, 27). In previous studies, we found that in rats cloxacillin was less effective in the treatment as well as the prophylaxis of endocarditis caused by a tolerant strain of *S. aureus* than in the treatment and prophylaxis of endocarditis caused by its nontolerant variant (36, 37).

In most studies on tolerance, β-lactam antibiotics were involved. Sometimes, bacteria that exhibit tolerance to β-lactam antibiotics are also tolerant to other antibiotics that inhibit cell wall synthesis. This was the case for the glycopeptide antibiotics vancomycin and teicoplanin, which interfere with peptidoglycan synthesis by inhibiting transglycosylase and probably also transpeptidase enzymes (17, 28). Although good bactericidal activities against gram-positive bacteria, including *S. aureus*, have been demonstrated in vitro for these antibiotics, treatment failures ascribed to tolerance have been reported (10, 13, 21, 30). However, no experimental studies on the effects of vancomycin and teicoplanin on tolerant *S. aureus* infections have yet been reported. For *Streptococcus sanguis*, Bernard et al. (3) reported that vancomycin might prevent endocarditis in rats challenged with a tolerant strain.

Daptomycin, a lipopeptide antibiotic with a spectrum of activity limited to gram-positive bacteria, inhibits the synthesis of peptidoglycan by preventing the active transport of cell wall amino acids (1). It has been shown to be rapidly bactericidal against *S. aureus* (31), and tolerance to this antibiotic has not yet been described.

To further elucidate the significance of tolerance in vivo, we investigated the influence of this phenomenon on the efficacy of treatment and prophylaxis with vancomycin, teicoplanin, and daptomycin of *S. aureus* endocarditis in rats.

MATERIALS AND METHODS

Antibiotics. Vancomycin and daptomycin were supplied by Eli Lilly & Co. (Indianapolis, Ind.); teicoplanin and rifampicin were supplied by Merrell Dow (Berkshire, England).

Microorganisms. *S. aureus* 372 tol, a clinical isolate from a bacteremic patient, is known to be tolerant to β-lactam antibiotics. Its nontolerant variant 372 ks was obtained from the parent strain by subculturing a few colonies of the original strain on Mueller-Hinton agar weekly for 60 days at 4°C. Both strains have been described previously (12, 36). The MIC and MBC of cloxacillin were 0.2 and >102.4 μg/ml, respectively, for 372 tol and 0.2 and 0.4 μg/ml, respectively, for 372 ks. Exposure of the tolerant strain to 30 μg of cloxacillin per ml caused no significant decrease in the number of bacteria over the first 6 h; at 24 h there was about a 1.5-$\log_{10}$-unit decrease.

* Corresponding author. Mailing address: Department of Infectious Diseases, University Hospital, Building 1, C5-P, P.O. 9600, NL-2300 RC Leiden, The Netherlands. Phone: 31-71-262620. Fax: 31-71-226605.

† Present address: Department of Medical Microbiology, University Hospital, Leiden, The Netherlands.

For the nontolerant strain, the same concentration of cloxacillin gave a reduction in bacterial numbers of 4 to 5 $\log_{10}$ CFU/ml at 24 h. Both strains had the same propensity to induce endocarditis in rats, as judged by 50% infective doses. These values were determined previously by infecting 44 rats with inocula ranging from 10^3 to 10^6 of either strain used in the present study (36). Furthermore, the strains did not differ in the early course of endocarditis or in the clearance of bacteria from the bloodstream, as shown previously (37). Stock cultures of these strains were lyophilized (26). In this state, tolerance was maintained in the original strain 372 tol. Fresh cultures were used for each experiment.

In vitro studies. The experiments described here were performed at the Department of Infectious Diseases, University Hospital Leiden, Leiden, The Netherlands. The MICs and MBCs of vancomycin, teicoplanin, daptomycin, and rifampin were determined by a broth macrodilution method with an inoculum of approximately 5×10^5 CFU/ml in Mueller-Hinton broth (MHB). This medium was supplemented with 25 μg of magnesium per ml and 50 μg of calcium per ml for determinations of daptomycin MICs and MBCs. To determine MBCs, aliquots of 50 μl were taken from each dilution of the antibiotics which did not exhibit turbidity after 24 h and were plated on diagnostic sensitivity test (DST) agar plates (Oxoid, London, England). Antibiotics were removed by centrifugation of the clear tubes at 1,500 × *g* for 10 min. Bacteria were resuspended in fresh MHB, and 50 μl was plated onto DST agar plates. After 48 h of incubation at 37°C, the viable bacteria were counted and were expressed as a percentage of the original inoculum. The MBC was defined as the lowest concentration of the antibiotic that killed at least 99.9% of the original inoculum after incubation for 24 h.

The susceptibilities of the strains to the antibiotics used in the study were also tested by plotting time-kill curves. Bacteria from an overnight culture were diluted in 10 ml of prewarmed MHB; calcium and magnesium were added when daptomycin was used. After preincubation at 37°C for 1.5 h to obtain logarithmic-phase growth, cultures with a density of about 5×10^6 CFU/ml were obtained. Antibiotics were added to final concentrations of 20 μg of vancomycin per ml, 10 μg of teicoplanin per ml, 10 μg of daptomycin per ml, and 2.5 μg of rifampin per ml. The concentrations chosen reflected the levels achievable in serum with the dosage regimens used in vivo. At the indicated intervals, 100-μl samples were taken and plated onto DST agar. After incubation for 24 to 48 h, the CFU was counted and plotted against time. Screening for the emergence of rifampin resistance after exposure to this antibiotic in vitro was performed by plating 100-μl samples onto DST agar plates containing 10 μg of rifampin per ml. After 24 h of incubation, the plates were examined for growth.

Studies in animals. Bacterial endocarditis was induced in female Wistar rats (weight, 220 to 250 g) by the method described by Héraïef et al. (15). At 72 h after catheterization, the animals were challenged with 10^6 CFU of the appropriate strain of *S. aureus*. This inoculum of the tolerant and the nontolerant strains induced endocarditis in all animals when the catheter was properly placed.

For treatment experiments, rats were randomly assigned to receive no treatment, vancomycin (100 mg/kg of body weight given intramuscularly [i.m.] every 12 h [b.i.d.]), teicoplanin (30 mg/kg given i.m. as a single dose or divided into two doses of 15 mg/kg b.i.d. preceded by a loading dose of 30 mg/kg), or daptomycin (10 mg/kg given subcutaneously [s.c.] as a single dose or divided into two doses of 5 mg/kg b.i.d.). Since preliminary in vitro studies showed enhanced bacterial killing when these antibiotics were combined with rifampin, some groups of rats were treated with a combination of vancomycin, teicoplanin, or daptomycin and rifampin (6 mg/kg given i.m.). Treatment was started 24 h after bacterial challenge and was continued for 5 days. Rats were killed 12 or 24 h after administration of the last dose of antibiotics. Rats receiving no antibiotics were killed when treatment was started.

For evaluation of prophylaxis, rats were injected with a single dose of vancomycin (10 or 50 mg/kg given i.m.), teicoplanin (1.2, 6, or 30 mg/kg given i.m.), or daptomycin (1 or 5 mg/kg given s.c.) 30 min before bacterial challenge with 10^6 CFU of one of the strains of *S. aureus*. The animals were killed 24 h after bacterial challenge. Controls receiving no prophylaxis were killed at the same time.

Vegetations were removed, weighed, and homogenized in 1 ml of sterile saline. The numbers of bacteria were quantified by serial dilution on DST agar plates and were expressed as $\log_{10}$ CFU per gram of vegetation. The sensitivity of this technique was 10 CFU per vegetation. Culture-negative specimens in the treatment group were assigned this value for calculation purposes and for comparison with other antibiotic regimens. Before sacrifice, blood samples were drawn and plated onto DST agar plates. Cultures lacking growth after 48 h of incubation were considered sterile.

Antibiotic levels in serum. Infected rats received one injection of vancomycin (10, 50, or 100 mg/kg given i.m.), teicoplanin (1.2, 6, or 30 mg/kg given i.m.), daptomycin (1, 5, or 10 mg/kg given s.c.), or rifampin (6 mg/kg given i.m.). After various intervals, blood samples were drawn from the orbital plexus. At least four rats were used for each sampling point. The concentrations of the antibiotics in serum were determined by an agar diffusion bioassay. *Bacillus subtilis* ATCC 6633 was the assay organism for vancomycin and teicoplanin; *Micrococcus luteus* ATCC 9341 was the assay organism for daptomycin and rifampin. The concentrations in serum were derived from standard curves prepared in normal pooled rat serum. The detection limit was 1.25 μg/ml for vancomycin, teicoplanin, and daptomycin and 0.25 μg/ml for rifampin. To determine whether accumulation of vancomycin, teicoplanin, or daptomycin occurred during treatment, additional determinations of the drug levels in serum were done on days 3 and 5 of treatment.

Susceptibilities of staphylococci in isolated material. Changes in the MICs of vancomycin, teicoplanin, and daptomycin for bacteria from isolated material were checked by an agar dilution technique (39) by using Mueller-Hinton agar supplemented as described above for daptomycin. Inocula of circa 10^4 CFU of the test organisms were applied with a 32-prong inoculator. Plates were examined for growth after 24 h of incubation at 37°C. For detection of rifampin resistance, aliquots of 50 μl of the vegetational homogenate isolated from antibiotic-treated animals were plated onto DST agar plates containing 10 μg of rifampicin per ml. Growth was examined after 24 h of incubation. To establish changes in the bactericidal effects of vancomycin, teicoplanin, and daptomycin, bacteria isolated from the vegetations obtained after treatment or prophylaxis were cultured for 24 h in MHB. Bacteria were diluted to approximately 5×10^6 CFU/ml and were exposed to 20 μg of vancomycin per ml, 10 μg of teicoplanin per ml, or 10 μg of daptomycin per ml. Survival percentages were determined after 24 h of incubation, as described above for the time-kill curves, and were compared with those found for the original strains.

Statistical analysis. Bacterial numbers in vegetations from rats infected with different strains and treated with the same antibiotic regimen were compared by an unpaired Student's *t* test. For multiple comparisons of the various treatment groups

TABLE 1. Susceptibilities of *S. aureus* 372 tol and 372 ks

Antibiotic	372 tol		372 ks	
	MIC (μg/ml)	MBC (μg/ml)	MIC (μg/ml)	MBC (μg/ml)
Vancomycin	0.5	>128	0.5	0.5
Teicoplanin	0.25	>128	0.25	0.5
Daptomycin	0.25	1.0	0.25	0.5
Rifampin	0.0125	0.5	0.0125	0.5

infected with the same strain, analysis of variance was used; this was followed by the Newman-Keuls test (11). Comparisons of the frequencies of sterilization of vegetations and blood cultures were made by Fisher's exact test. For multiple comparisons of the frequencies of sterilization, Bonferroni's adjustment was used (23). *P* values of <0.05 and *P* values determined by Bonferroni's correction were considered significant.

RESULTS

In vitro studies. The MICs and MBCs of vancomycin, teicoplanin, daptomycin, and rifampin for 372 tol and 372 ks are given in Table 1. When a MBC/MIC ratio of ≥ 32 was used as the criterion for tolerance, strain 372 tol was tolerant to vancomycin and teicoplanin but not daptomycin. Rifampin was not bactericidal for either strain.

The time-kill curves are shown in Fig. 1. For strain 372 tol, vancomycin and teicoplanin did not significantly decrease the

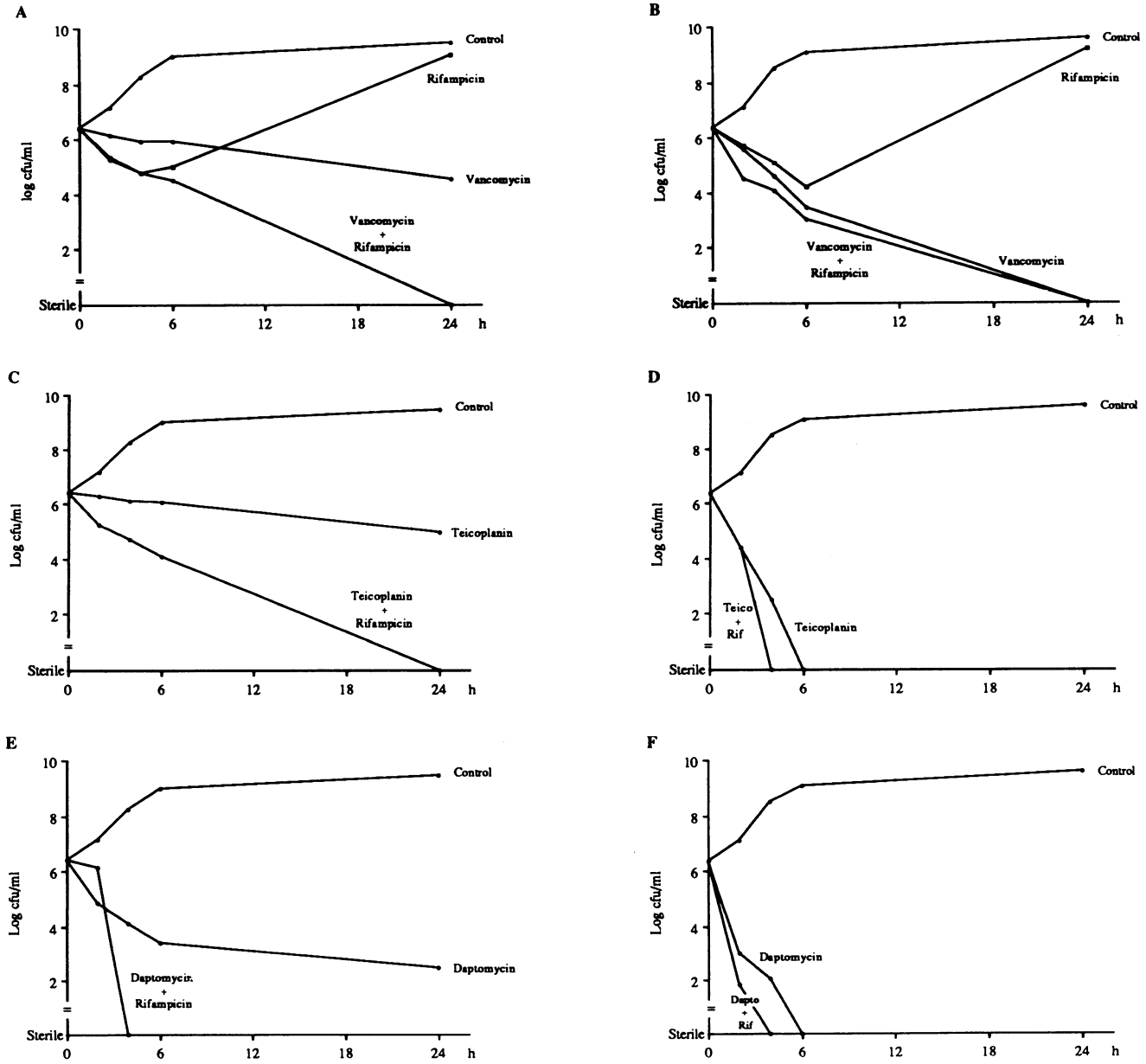

FIG. 1. Killing of *S. aureus* 372 tol (A, C, E) and 372 ks (B, D, F) by vancomycin (20 μg/ml), teicoplanin (10 μg/ml), daptomycin (10 μg/ml), or rifampin (2.5 μg/ml) alone or in combination.

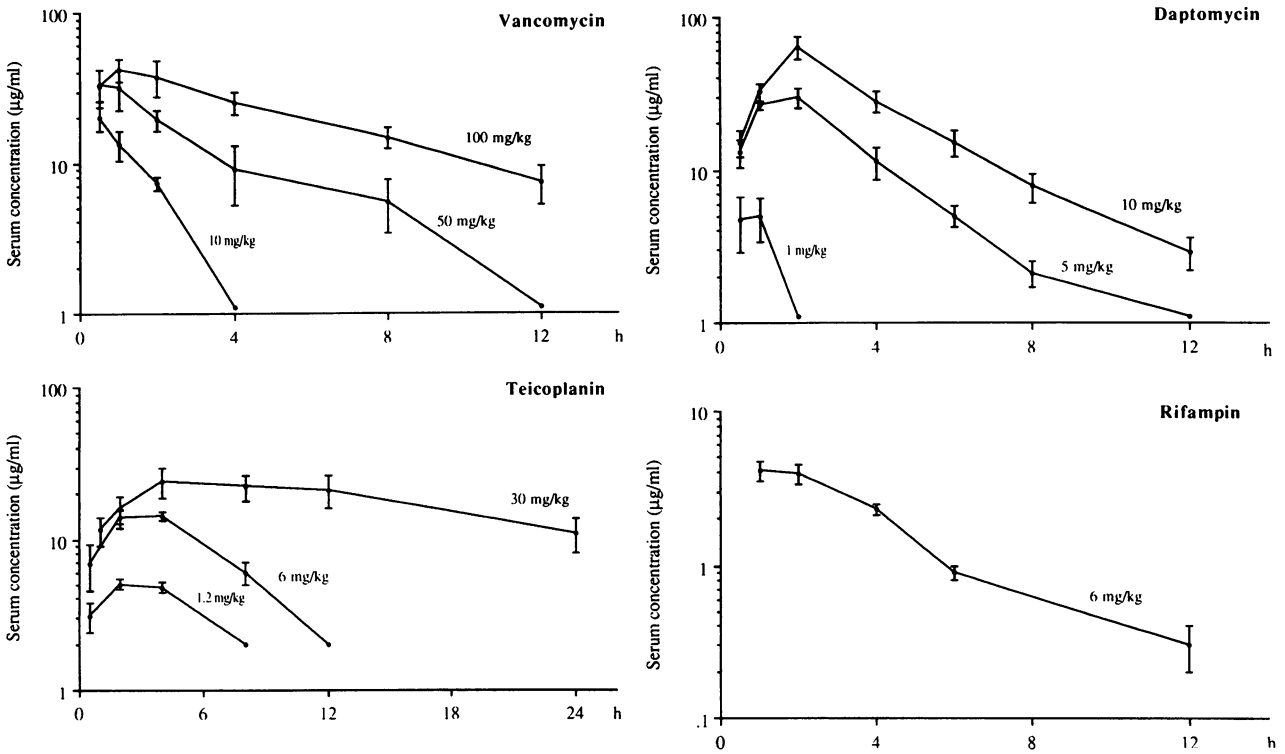

FIG. 2. Concentrations (mean ± standard deviation) of antibiotics in rat sera after administration of vancomycin, teicoplanin, daptomycin, and rifampin at the indicated doses. Each point is the mean value for at least four rats.

number of bacteria during the first 6 h, while at 24 h the decrease in the number of CFU per milliliter was less than 2.5 $\log_{10}$ units. In contrast, both antibiotics rapidly and significantly killed strain 372 ks, with a reduction in bacterial numbers of approximately 3 $\log_{10}$ units during the first 6 h, while at 24 h, the bacteria could no longer be cultured. These results are in accordance with the MBC/MIC ratios of these antibiotics for the respective strains. Daptomycin was bactericidal against both 372 tol and 372 ks, but the decrease in bacterial numbers was more pronounced for the latter strain. The combination of each of the antibiotics with rifampin enhanced the killing of both strains. Exposure of both strains to rifampin alone resulted in a reduction in bacterial numbers of about 2 $\log_{10}$ units within 6 h, but regrowth of the bacteria was found at 24 h; moreover, rifampin-resistant organisms emerged in subculture plates containing rifampin (10 μg/ml). During exposure to rifampin with the other antibiotics, no resistance developed.

Antibiotic levels in serum. The levels of the drugs in serum after one intramuscular injection of vancomycin, teicoplanin, or rifampin and one subcutaneous injection of daptomycin at the various dose levels are shown in Fig. 2. Peak and trough levels in serum after 3 and 5 days of treatment were comparable to those after the first injection, indicating that no accumulation of the drug occurred during treatment (data not shown).

Treatment of endocarditis. Mortality before the beginning of treatment was not significantly different between the two strains, i.e., 7 of 141 rats for 372 tol and 8 of 137 rats for 372 ks. Furthermore, there was no significant difference between the antibiotics in mortality during treatment. Mortality varied from of 0 to 20% (data not shown). Only rats that survived the entire treatment period were included in the calculations.

The results of the various therapeutic regimens are shown in Table 2. For both strains, all treatment regimens reduced bacterial counts in vegetations relative to those in the vegetations of the control groups ($P < 0.001$). Moreover, the efficacy of each antibiotic regimen was about the same for strains 372 tol and 372 ks. Comparisons between groups infected with the same strain revealed that, for both strains, daptomycin was significantly more effective than vancomycin or teicoplanin in reducing bacterial numbers in the vegetations when the drugs were given b.i.d. ($P < 0.05$). For both strains, teicoplanin at a dose of 30 mg/kg and daptomycin at a dose of 10 mg/kg were more effective when given in two doses b.i.d. than once daily. In comparison with vancomycin or teicoplanin alone, the combination of each drug with rifampin resulted in a significantly larger reduction in bacterial densities ($P < 0.025$ and $P < 0.0005$ for vancomycin and teicoplanin, respectively). Use of the combination of rifampin with daptomycin did not result in a higher efficacy compared with the use of daptomycin alone.

The same trends were observed for the sterilization of vegetations (Table 2). All antibiotics yielded about the same rate of sterilization in rats infected with 372 tol as in those infected with 372 ks. Combinations with rifampin were, in general, more effective than the single agents in rendering vegetations sterile, but the differences were not significant.

After most treatment regimens, 80 to 100% of the rats infected with either strain had sterile blood cultures. However, rifampin (6 mg/kg b.i.d.), daptomycin (10 mg/kg daily), and teicoplanin (30 mg/kg daily) were less effective than the other regimens in this respect (Table 2).

Prophylaxis of endocarditis. The effects of prophylaxis with the various antibiotic regimens of endocarditis induced with strains 372 tol and 372 ks are summarized in Table 3. Vancomycin at a dose of 50 mg/kg, teicoplanin at a dose of 30 or 6

TABLE 2. Treatment of endocarditis in rats caused by *S. aureus* 372 tol and 372 ks

Treatment[a]	Mean ± SD $\log_{10}$ CFU/g of vegetation (no. of rats with sterile vegetations/total no.)		No. of rats with sterile blood cultures/total no.	
	372 tol	372 ks	372 tol	372 ks
None	9.64 ± 0.44 (0/13)	9.89 ± 0.58 (0/13)	0/13	0/13
Vancomycin (100 mg/kg b.i.d.)	5.55 ± 1.75 (1/14)	5.24 ± 2.01 (3/11)	14/14	10/11
Vancomycin-rifampin	3.91 ± 1.50 (3/12)	3.54 ± 1.80 (7/11)	11/12	11/11
Teicoplanin (15 mg/kg b.i.d.)	6.95 ± 2.57 (2/16)	6.83 ± 2.66 (4/22)	13/16	11/22
Teicoplanin-rifampin	3.39 ± 0.96 (4/11)	3.57 ± 1.50 (6/12)	11/11	12/12
Teicoplanin (30 mg/kg daily)	8.61 ± 0.38 (1/13)	7.77 ± 1.97 (1/12)	4/13	4/12
Daptomycin (5 mg/kg b.i.d.)	3.87 ± 1.82 (8/15)	3.43 ± 0.94 (4/13)	13/15	10/13
Daptomycin-rifampin	3.77 ± 1.52 (5/11)	3.28 ± 1.31 (7/11)	11/11	11/11
Daptomycin (10 mg/kg daily)	6.80 ± 2.48 (1/10)	7.32 ± 2.64 (1/10)	4/10	6/10
Rifampin (6 mg/kg b.i.d.)	7.91 ± 1.54 (0/6)	7.56 ± 1.56 (0/8)	3/6	4/8

[a] Rats received the different antibiotic regimens for 5 days. Only rats that survived the entire treatment period were included in calculations. There was no statistical difference in bacterial densities in vegetations between groups of rats infected with 372 tol and 372 ks for any treatment regimen.

mg/kg, and daptomycin at a dose of 5 mg/kg were equally effective in preventing endocarditis in rats challenged with 372 tol and 372 ks. However, vancomycin at the lower dose of 10 mg/kg as well as teicoplanin at the lowest dose of 1.2 mg/kg was significantly less effective against 372 tol than against 372 ks (372 tol versus 372 ks, $P < 0.015$ for both regimens). At a lower dose of 1 mg of daptomycin per kg, the proportion of rats with sterile vegetations was lower among those infected with 372 tol than among those infected with 372 ks, but this difference was not significant ($P = 0.11$). Comparison of the various prophylactic regimens within one strain showed that for 372 tol, vancomycin was less protective at a dose of 10 mg/kg than at a dose of 50 mg/kg ($P < 0.03$). Teicoplanin at a low dose of 1.2 mg/kg was also less effective in preventing infection with the tolerant strain than were the higher doses of this antibiotic ($P < 0.0015$). In contrast, for 372 ks all antibiotic regimens afforded almost full protection, even at the lowest doses. The numbers of bacteria in vegetations from rats infected with either strain, despite antibiotic prophylaxis, were lower than those in the vegetations from controls at 24 h (Table 3). Statistical differences between the bacterial densities in vegetations from rats infected with 372 tol and 372 ks could not be calculated, because only small numbers of rats had infected vegetations after prophylaxis.

In general, all prophylactic regimens yielded approximately similar proportions of rats with sterile blood cultures and sterile vegetations (Table 3).

Susceptibilities of bacteria after exposure to antibiotics in vivo. For both strains, the MICs of vancomycin, teicoplanin, and daptomycin for bacteria isolated from vegetations were similar to or only twice the values for the original strains. After infection with 372 tol, isolates resistant to rifampin were recovered from 3 of 6 rats treated with this agent and from 3 of 12 rats treated with the combination of rifampin and vancomycin. Six of 8 rats treated with rifampin alone and 3 of 12 rats treated with the combination of rifampin and vancomycin were resistant to rifampin after infection with 372 ks. Other combinations with rifampin did not yield isolates resistant to rifampin.

Bacteria isolated after treatment or prophylaxis from rats infected with either strain were exposed to 20 μg of vancomycin per ml, 10 μg of teicoplanin per ml, and 10 μg of

TABLE 3. Results of prophylaxis in rats challenged with *S. aureus* 372 tol and 372 ks

Regimen[a]	No. of rats with sterile vegetations/total no.		Log CFU/g of vegetation (mean ± SD)		No. of rats with sterile blood cultures/total no.	
	372 tol	372 ks	372 tol	372 ks	372 tol	372 ks
Control	0/12	0/13	10.13 ± 0.51	9.99 ± 0.38	0/12	0/13
Vancomycin						
50 mg/kg	14/16	14/17	9.19, 6.12	3.54 ± 0.28	14/16	17/17
10 mg/kg	8/16[b]	13/14	6.89 ± 1.68	6.17	7/16[b]	13/14
Teicoplanin						
30 mg/kg	14/15	11/12	3.10	4.11	15/15	11/12
6 mg/kg	14/16	12/14	5.04, 3.70	2.70, 3.48	15/16	14/14
1.2 mg/kg	2/10[c]	8/10	4.92 ± 2.01	4.30, 4.48	5/10[c]	10/10
Daptomycin						
5 mg/kg	10/11	11/12	4.24	3.00	8/11	12/12
1 mg/kg	10/19	16/21	5.25 ± 2.19	7.46 ± 2.25	10/19	20/21

[a] Antibiotics were given 30 min before bacterial challenge. Rats were killed 24 h after bacterial challenge. Blood for cultures was obtained before sacrifice.
[b] For strain 372 tol versus strain 372 ks, $P < 0.015$.
[c] For strain 372 tol versus strain 372 ks, $P < 0.02$.

daptomycin per ml. Survival percentages of these microorganisms were not changed after in vivo exposure to the antibiotics (data not shown).

DISCUSSION

In the study described here, the efficacies of vancomycin, teicoplanin, and daptomycin for the treatment and prophylaxis of *S. aureus* endocarditis in rats caused by a tolerant strain and its nontolerant variant were compared. It was found that the cloxacillin-tolerant strain of *S. aureus* (372 tol) was also tolerant to vancomycin and teicoplanin, with MBC/MIC ratios of more than 256 and slower rates of killing during exposure to these antibiotics. Interestingly, strain 372 tol was not tolerant to daptomycin, although it was killed more slowly than the nontolerant strain 372 ks. However, these in vitro susceptibilities did not predict treatment results since there was no statistical difference in the bacterial densities in vegetations between rats infected with either 372 tol or 372 ks after 5 days of treatment with vancomycin, teicoplanin, or daptomycin at any dose. Thus, tolerance to vancomycin or teicoplanin did not influence the treatment results.

In contrast, the proportion of rats with sterile vegetations receiving prophylaxis with vancomycin or teicoplanin at the lowest dose was lower for those infected with 372 tol than for those infected with 372 ks. Since the tolerant and the nontolerant strains did not differ in their virulences, as judged by 50% infective doses, the early course of the infection, and clearance of bacteria from the bloodstream, it is most likely that the observed differences in the efficacies of prophylaxis were due to tolerance. Furthermore, in other studies we found that prophylaxis with antibiotics that are not active against the bacterial wall, such as gentamicin and ciprofloxacin, was equally effective for the tolerant and the nontolerant strains (37, 38). The similar efficacies of antibiotics that are not affected by tolerance against both strains makes it unlikely that properties other than tolerance account for the differences between the two strains in the efficacies of prophylaxis with vancomycin and teicoplanin. At the lower dose of daptomycin, the proportion of rats with sterile vegetations challenged with 372 tol was also lower than that after challenge with 372 ks, but this difference was not significant. Higher doses of all three antibiotics afforded almost full protection from 372 tol. Apparently, the higher doses, which gave a longer exposure to the antibiotic because of a prolonged adequate level in serum, had an effect even on the tolerant bacteria that was sufficient to prevent endocarditis, despite the slower killing rate. However, bacterial densities in the vegetations of rats that were infected with strain 372 tol, despite prophylaxis with vancomycin at a dose of 50 mg/kg, were higher than in those of rats that were infected with strain 372 ks. This could have been due to the slower killing of tolerant bacteria by vancomycin within the vegetations. Since only two and three rats were infected after prophylaxis with strains 372 tol and 372 ks, respectively, statistical analysis of these data could not be done. Thus, interpretation of the results must be done with caution.

Daptomycin was more effective than vancomycin or teicoplanin in reducing bacterial numbers of both strains in the vegetations. For daptomycin as well as teicoplanin, administration of two daily doses was more effective than administration of the same total dose once daily. These results are in accordance with the findings of other studies on the treatment of experimental *S. aureus* endocarditis with these antibiotics (5, 19). Teicoplanin is poorly distributed throughout vegetations, as has been shown by autoradiography (7). The same could be true for the structurally related compound vancomycin. In contrast, daptomycin is homogeneously distributed throughout the vegetations (6). This difference in penetration could be one of the explanations for the greater efficacy of daptomycin in comparison with those of the glycopeptide antibiotics.

It has been shown that the combination of rifampin with vancomycin or teicoplanin may improve the results of treatment of staphylococcal endocarditis in animals (2, 34). In clinical studies, no benefit of the addition of rifampin to vancomycin or teicoplanin has been reported (22, 35). No in vivo data on the combination of daptomycin and rifampicin are available. However, in vitro synergy as well as antagonism have been reported for the combination of vancomycin, teicoplanin, or daptomycin with rifampin (8, 33, 40). For the strains used in the present study, the combination of rifampin with vancomycin, teicoplanin, or daptomycin was synergistic in vitro. Moreover, the combination of rifampin and vancomycin or teicoplanin improved the results of treatment for the tolerant as well as the nontolerant strain. Daptomycin in combination with rifampin was as effective as daptomycin alone in reducing bacterial numbers in vegetations infected with either strain, however. Treatment with rifampin as a single agent significantly reduced bacterial numbers in the vegetations, but its use as a single agent is limited because of the rapid development of resistance. Rifampin resistance was not encountered in the present study when rifampin was administered in combination with teicoplanin or daptomycin. Rifampin resistance occurred in some rats treated with the combination of rifampin and vancomycin. This has also been described in patients treated with this combination regimen and in experimental models of infection (9, 29).

An increase in the MIC of teicoplanin during treatment has been reported in patients infected with *S. aureus* (18, 24), but in our study in rats, such an increase was not found after 5 days of treatment. Survival percentages for microorganisms isolated from the vegetations of rats infected with the tolerant strain did not change after in vivo exposure to the antibiotics used in the study. Thus, the results were not influenced by a loss of tolerance. Furthermore, the bacteria isolated from animals infected with the nontolerant strain remained so.

In conclusion, tolerance did not influence treatment of *S. aureus* endocarditis in rats with vancomycin, teicoplanin, or daptomycin, but the prophylactic efficacies of these agents were reduced by the tolerance phenomenon. However, extrapolation of these experimental data to the clinical situation must be done with caution, especially since tolerance had a significant effect only when lower doses of vancomycin or teicoplanin were used.

ACKNOWLEDGMENTS

This work was supported by The Netherlands Heart Foundation (grant 87.023).

We thank R. van Furth for critical reading of the manuscript and H. Chander for technical assistance.

REFERENCES

1. **Allen, N. E., W. E. Alborn, and J. N. Hobbs, Jr.** 1991. Inhibition of membrane potential-dependent amino acid transport by daptomycin. Antimicrob. Agents Chemother. **35**:2639–2642.
2. **Bayer, A. S., and K. Lam.** 1985. Efficacy of vancomycin plus rifampin in experimental aortic valve endocarditis due to methicillin-resistant *Staphylococcus aureus*: in vitro-in vivo correlations. J. Infect. Dis. **151**:157–165.
3. **Bernard, J. P., P. Francioli, and M. P. Glauser.** 1981. Vancomycin prophylaxis of experimental *Streptococcus sanguis*: inhibition of bacterial adherence rather than bacterial killing. J. Clin. Invest. **68**:1113–1116.
4. **Brennan, R. O., and D. T. Durack.** 1983. Therapeutic significance

of penicillin tolerance in experimental streptococcal endocarditis. Antimicrob. Agents Chemother. **23**:273–277.

5. **Cantoni, L., M. P. Glauser, and J. Bille.** 1990. Comparative efficacy of daptomycin, vancomycin, and cloxacillin for the treatment of *Staphylococcus aureus* endocarditis in rats and role of test conditions in this determination. Antimicrob. Agents Chemother. **34**:2348–2353.
6. **Caron, F., M. D. Kitzis, L. Gutmann, A. C. Cremieux, B. Maziere, J. M. Vallois, A. Saleh-Mghir, J. F. Lemeland, and C. Carbon.** 1992. Daptomycin or teicoplanin in combination with gentamicin for treatment of experimental endocarditis due to a highly glycopeptide-resistant isolate of *Enterococcus faecium*. Antimicrob. Agents Chemother. **36**:2611–2616.
7. **Cremieux, A. C., B. Maziere, J. M. Valois, M. Ottaviani, A. Azancot, H. Raffoul, A. Bouvet, J. J. Pocidalo, and C. Carbon.** 1989. Evaluation of antibiotic diffusion into cardiac vegetations by qualitative autoradiography. J. Infect. Dis. **159**:938–944.
8. **Debbia, E., A. Pesce, and G. C. Schito.** 1988. In vitro activity of LY146032 alone and in combination with other antibiotics against gram-positive bacteria. Antimicrob. Agents Chemother. **32**:279–281.
9. **Dworkin, R., G. Modin, S. Kunz, R. Rich, O. Zak, and M. Sande.** 1990. Comparative efficacies of ciprofloxacin, pefloxacin, and vancomycin in combination with rifampin in a rat model of methicillin-resistant *Staphylococcus aureus* chronic osteomyelitis. Antimicrob. Agents Chemother. **34**:1014–1016.
10. **Faville, R. J., Jr., D. E. Zaske, E. L. Kaplan, K. Crossley, L. D. Sabath, and P. G. Quie.** 1978. *Staphylococcus aureus* endocarditis. Combined therapy with vancomycin and rifampicin. JAMA **240**: 1963–1965.
11. **Godfrey, K.** 1985. Comparing the mean of several groups. N. Engl. J. Med. **23**:1450–1456.
12. **Goessens, W. H. F., P. Formijne, and M. F. Michel.** 1984. Responses of tolerant and nontolerant *Staphylococcus aureus* strains to methicillin treatment in an experimental infection in mice. Antimicrob. Agents Chemother **26**:829–834.
13. **Gopal, V., A. L. Bisno, and F. J. Silverblatt.** 1976. Failure of vancomycin treatment in *Staphylococcus aureus* endocarditis. JAMA **236**:1604–1606.
14. **Handwerger, S., and A. Tomasz.** 1985. Antibiotic tolerance among clinical isolates of bacteria. Rev. Infect. Dis. **7**:368–386.
15. **Héraïef, M. P. Glauser, and L. R. Freedman.** 1982. Natural history of aortic valve endocarditis in rats. Infect. Immun. **37**:127–131.
16. **Hess, J., J. Dankert, and D. Durack.** 1983. Significance of penicillin tolerance in vivo: prevention of experimental *Streptococcus sanguis* endocarditis. J. Antimicrob. Chemother. **11**:555–564.
17. **Jordan, D. C., and H. D. C. Mallory.** 1964. Site of action of vancomycin on *Staphylococcus aureus*, p. 489–494. Antimicrob. Agents Chemother. 1963.
18. **Kaatz, G. W., S. M. Seo, N. J. Dorman, and S. A. Lerner.** 1990. Emergence of teicoplanin resistance during therapy of *Staphylococcus aureus* endocarditis. J. Infect. Dis. **162**:103–108.
19. **Kaatz, G. W., S. M. Seo, V. N. Reddy, E. M. Bailey, and M. J. Rybak.** 1990. Daptomycin compared with teicoplanin and vancomycin for therapy of experimental *Staphylococcus aureus* endocarditis. Antimicrob. Agents Chemother. **34**:2081–2085.
20. **Kim, K., and A. S. Bayer.** 1987. Significance of in-vitro penicillin tolerance in experimental enterococcal endocarditis. J. Antimicrob. Chemother. **19**:475–485.
21. **Leport, C., C. Peronne, P. Massip, P. Canton, P. Leclercq, E. Bernard, P. Lutun, J. J. Garaud, and J. L. Vilde.** 1989. Evaluation of teicoplanin for treatment of endocarditis caused by gram-positive cocci in 20 patients. Antimicrob. Agents Chemother. **33**:871–876.
22. **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.
23. **MacArthur, R. D., and G. G. Jackson.** 1984. An evaluation of the use of statistical methodology in the Journal of Infectious Diseases. J. Infect. Dis. **149**:349–354.
24. **Manquat, G., J. Croize, J. P. Stahl, M. Meyran, P. Hirtz, and M. Micoud.** 1992. Failure of teicoplanin treatment associated with an increase in MIC during therapy of *Staphylococcus aureus* septicaemia. J. Antimicrob. Chemother. **29**:731–732.
25. **Meeson, J., A. A. McColm, P. Acred, and D. Greenwood.** 1990. Differential response to benzylpenicillin in vivo of tolerant and non-tolerant variants of *Streptococcus sanguis* II. J. Antimicrob. Chemother. **25**:103–109.
26. **Michel, M. F., and W. B. van Leeuwen.** 1989. Degree and stability of tolerance to penicillin in *Streptococcus pyogenes*. Eur. J. Clin. Microbiol. Infect. Dis. **8**:225–232.
27. **Pujadas, R., E. Escriva, J. Jane, J. Argimon, F. Fernandez, P. Fava, M. Galera, and J. Garau.** 1990. Tolerance and efficacy of parenterally administered penicillin-streptomycin and orally administered amoxicillin or penicillin V for prophylaxis of experimentally induced streptococcal endocarditis. Antimicrob. Agents Chemother. **34**:321–325.
28. **Reynolds, P. E.** 1989. Structure, biochemistry and mode of action of glycopeptide antibiotics. Eur. J. Clin. Microbiol. Infect. Dis. **8**:943–950.
29. **Simon, G. L., R. H. Smith, and M. A. Sande.** 1983. Emergence of rifampin-resistant strains of *Staphylococcus aureus* during combination therapy with vancomycin and rifampin; a report of two cases. Rev. Infect. Dis. **5**(Suppl. 3):407–411.
30. **Sorrell, T. C., D. R. Packman, S. Shanker, M. Foldes, and R. Munro.** 1982. Vancomycin therapy for methicillin-resistant *Staphylococcus aureus*. Ann. Intern. Med. **97**:344–350.
31. **Stratton, C. W., C. Liu, and L. S. Weeks.** 1987. Activity of LY146032 compared with that of methicillin, cefazolin, cefamandole, cefuroxin, ciprofloxacin, and vancomycin against staphylococci as determined by kill-kinetic studies. Antimicrob. Agent Chemother. **31**:1210–1215.
32. **Tomasz, A., A. Albino, and E. Zanati.** 1970. Multiple antibiotic resistance in a bacterium with suppressed autolytic system. Nature (London) **227**:138–140.
33. **Tuazon, C. U., and H. Miller.** 1984. Comparative in vitro activities of teichomycin and vancomycin alone and in combination with rifampin and aminoglycosides against staphylococci and enterococci. Antimicrob. Agents Chemother. **25**:411–412.
34. **Tuazon, C. U., and D. Washburn.** 1987. Teicoplanin and rifampin singly and in combination in the treatment of experimental *Staphylococcus epidermidis* endocarditis in the rabbit model. J. Antimicrob. Chemother. **20**:233–237.
35. **Van Der Auwera, P., F. Meunier-Carpentier, and J. Klastersky.** 1985. Clinical study of combination therapy with oxacillin and rifampicin for staphylococcal infections. Rev. Infect. Dis. **5**(Suppl. 3):515–522.
36. **Voorn, G. P., J. Thompson, W. H. F. Goessens, W. C. Schmal-Bauer, P. H. M. Broeders, and M. F. Michel.** 1991. Role of tolerance in cloxacillin treatment of experimental *Staphylococcus aureus* endocarditis. J. Infect. Dis. **163**:640–643.
37. **Voorn, G. P., J. Thompson, W. H. F. Goessens, W. C. Schmal-Bauer, P. H. M. Broeders, and M. F. Michel.** 1992. Role of tolerance in cloxacillin prophylaxis of experimental *Staphylococcus aureus* endocarditis. J. Infect. Dis. **166**:169–173.
38. **Voorn, G. P., J. Thompson, W. H. F. Goessens, W. C. Schmal-Bauer, P. H. M. Broeders, and M. F. Michel.** Efficacy of ciprofloxacin in treatment and prophylaxis of experimental *Staphylococcus aureus* endocarditis caused by a cloxacillin-tolerant strain and its nontolerant variant. J. Antimicrob. Chemother, in press.
39. **Washington, J. A., and V. L. Sutter.** 1980. Dilution susceptibility test: agar and macro-broth dilution procedures, p. 453–458. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and J. P. Truant (ed.), Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
40. **Watanakunakorn, C., and J. C. Guerriero.** 1981. Interaction between vancomycin and rifampin against *Staphylococcus aureus*. Antimicrob. Agents Chemother. **19**:1089–1091.