ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 1995, p. 2289–2294 Vol. 39, No. 10
0066-4804/95/$04.00+0

Vancomycin or Vancomycin plus Netilmicin for Methicillin- and Gentamicin-Resistant *Staphylococcus aureus* Aortic Valve Experimental Endocarditis

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Received 28 November 1994/Returned for modification 9 February 1995/Accepted 30 June 1995

Using a rabbit model of aortic valve endocarditis, we studied the efficacy of vancomycin alone or in combination with netilmicin and/or rifampin against a methicillin- and gentamicin-resistant strain of *Staphylococcus aureus* (MGRSA). Antibiotics were given for 6 or 12 days, as follows: vancomycin (15 mg/kg of body weight every 12 h [BID] intravenously), vancomycin plus netilmicin (2.5 mg/kg BID intramuscularly), vancomycin plus rifampin (10 mg/kg BID intramuscularly), and vancomycin plus netilmicin plus rifampin at the same routes, dosages, and schedules mentioned above. Netilmicin was given to two additional groups at a higher dosage (6 mg/kg every 24 h intramuscularly) alone or in combination with vancomycin (15 mg/kg BID intravenously) for 12 days. All regimens resulted in undetectable bacterial counts in a significant proportion of vegetations (except netilmicin alone) or reduced the bacterial counts in the vegetations compared with the counts in the untreated controls ($P < 0.01$ to $P < 0.001$). No resistance to rifampin or netilmicin developed during therapy. It is concluded that in the treatment of experimental aortic valve endocarditis caused by MGRSA (i) vancomycin as monotherapy is as efficacious as the triple combination, (ii) the addition of netilmicin (once daily or BID) to vancomycin does not improve the efficacy of the latter antibiotic, even in the presence of rifampin, and (iii) a 12-day course is more effective than a 6-day one, but not at a statistically significant level.

Staphylococcus aureus is the second most common cause of infective endocarditis. It affects either native (25 to 35%) or prosthetic valves and is the most common organism (70%) recovered from intravenous drug addicts with endocarditis (23). Methicillin-resistant *S. aureus* (MRSA) strains have spread worldwide, and the frequency of their isolation has recently increased steeply (5, 12). MRSA isolates should be considered resistant to all β-lactams, and very often these organisms are cross resistant to gentamicin; however, they are uniformly susceptible to vancomycin and are usually susceptible to rifampin (5, 23).

Vancomycin is clearly the drug of choice for the treatment of infections caused by MRSA as well as an alternative to beta-lactam antibiotics for the treatment of methicillin-susceptible *S. aureus* infections, particularly in patients allergic to β-lactams (8, 24). Nevertheless, there have been several reports of therapeutic failures in patients with *S. aureus* endocarditis treated with vancomycin as monotherapy (18, 33, 35). Even in animal models, vancomycin has been found to be "surprisingly unsuccessful" against MRSA endocarditis (9). On the other hand, the therapeutic role of rifampin in MRSA endocarditis is controversial (4), and today the majority of MRSA endocarditis strains are also gentamicin resistant (23). Because netilmicin (i) has been found to be active in vitro against MRSA strains cross resistant to gentamicin (16a), (ii) it possesses decreased nephrotoxic potential in comparison with that of gentamicin (11, 22, 26, 31), and (iii) it has advantageous pharmacokinetics in cardiac valves (13) because of the time that its concentration in the heart valve is greater than the MIC at which 90% of *S. aureus* strains are inhibited is seven times longer than that of gentamicin (3.5 versus 0.5 h, respectively), we designed the present study in order to compare the therapeutic efficacy of vancomycin, alone or in combination with netilmicin and/or rifampin, in the rabbit model of aortic valve endocarditis caused by a MRSA strain which was also resistant to gentamicin (MGRSA) but which was susceptible to netilmicin. To our knowledge no study evaluating the efficacies of vancomycin and netilmicin as well as the efficacy of the combination with rifampin against MGRSA endocarditis has yet been published.

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MATERIALS AND METHODS

Microorganism. The MGRSA strain used in the study was a clinical isolate obtained from a patient with septicemia. The strain was identified as MGRSA in vitro after testing its susceptibilities to oxacillin and gentamicin by the Kirby-Bauer method after using 1- and 10-μg susceptibility disks, respectively (15). The gentamicin resistance mechanism of the strain was determined after applying the aminoglycoside resistance patterns to 12 aminoglycosides by a disk susceptibility test described by Miller et al. (28).

Antibiotic susceptibility testing. The MICs and MBCs of oxacillin (plus 2% NaCl), vancomycin, gentamicin, netilmicin, and rifampin were determined initially after applying ready-made microdilution plates (MD; gram positive and GRMK2B; Sensititre Ltd., West Sussex, England) at an inoculum of $\simeq 5 \times 10^5$ CFU/ml. Since the concentration range that can be tested with the ready-made microdilution plates is quite narrow, a manual microdilution technique was used to determine the exact MICs of oxacillin, gentamicin, netilmicin, and rifampin in volumes of 0.05 ml of cation-supplemented Mueller-Hinton broth (BBL Microbiology Systems). Therefore, the concentration ranges of the antibiotics tested were from 0.25 to 512 μg/ml. Susceptibilities to the same agents at an inoculum of $\simeq 5 \times 10^7$ CFU/ml were also determined, because the latter number of bacteria simulates more closely the pretreatment bacterial load in the vegetation. The MIC of each drug was defined as the lowest concentration causing no visible

turbidity after 18 h of incubation at 35°C. The MBC was determined by quantitative subculture of 50 μl from each clear well onto antibiotic free-blood agar plates and was defined as the lowest concentration that reduced the number of organisms by ≥99.9% of the initial inoculum.

The MICs and the MBCs for the surviving bacteria in the vegetations after the end of therapy and those for the parent strain were determined in parallel at inocula of $\simeq 5 \times 10^5$ and $\simeq 5 \times 10^7$ CFU/ml in order to detect any possible emergence of resistance during treatment.

Interaction studies. The in vitro bactericidal effects of the study drugs were assessed by the time-kill curve method (14). An overnight culture in Mueller-Hinton broth was used to prepare inocula at $\simeq 5 \times 10^5$ and $\simeq 5 \times 10^7$ CFU/ml. The final antibiotic concentrations tested in all interaction experiments were equivalent to the MBCs, as determined with an inoculum of $\simeq 5 \times 10^5$ CFU/ml. An additional killing curve study with an inoculum of $\simeq 5 \times 10^7$ CFU/ml and final antibiotic concentrations equivalent to the MBCs, as determined with an inoculum of $\simeq 5 \times 10^7$ CFU/ml, was also performed. Interaction studies were performed in duplicate. The MICs and MBCs for the surviving bacteria were also determined after 24 h of incubation. Bactericidal synergy was considered if the drug combination caused (i) a decrease of ≥ 2 $\log_{10}$ in the number of CFU per milliliter after 6 and/or 24 h of subculture compared with the effect of the single most effective drug used alone, and (ii) a final inoculum of the surviving bacteria after 24 h lower than the initial inoculum at time zero.

Antibiotics. Vancomycin was supplied by Eli Lilly Co., Indianapolis, Ind.; netilmicin was supplied by Schering Plough Corp., Bloomfield, N.J.; and rifampin was supplied by G. Lepetit (Marion Merrell Dow, S.p.A., Milan, Italy).

Endocarditis model. For the production of endocarditis the rabbit model described by Perlman and Freedman (32) was applied. A total of 189 New Zealand White rabbits weighing 2.5 to 3 kg each were anesthetized by intramuscular injection of ketamine hydrochloride (15 mg/kg) of body weight). The left carotid artery was exposed in the neck and was cannulated with a polyethylene catheter. The tip of the catheter was placed across the aortic valve into the left ventricle, and the proximal end was then secured in place in the neck for the duration of the experiment. Twenty-four hours after catheterization 10^7 CFU of the infecting strain suspended in 1 ml of saline was injected via the marginal ear vein. Blood samples for quantitative culture were taken 24 h thereafter. Presumptive confirmation of the induction of MGRSA endocarditis was based on positive results of blood cultures. Ultimate confirmation of bacterial endocarditis was based on macroscopic observation (vegetations and correct placement of the catheter across the aortic valve) and bacteriological data obtained at autopsy. Only data for rabbits with an initial blood culture positive for the infecting strain and in which the catheter was positioned across the aortic valve at autopsy were included in the results. Of the latter rabbits, data for those that were untreated controls were included in the results, regardless of the time of death, but those that had received antibiotics were included only if they had received four or more doses of antibiotics. Nevertheless, all treated animals that died after the initiation of therapy were included in the calculation of survival rate. Because all untreated rabbits with positive blood cultures at 24 h postinoculation had positive vegetation cultures with high inocula, rabbits in any treatment group with a positive pretherapy blood culture and sterile vegetation posttherapy were considered to be successfully treated.

Treatment was started 24 h after bacterial challenge and was continued for 6 or 12 days. The 12-day regimen was chosen in order to determine whether a more prolonged therapy would improve efficacy. Rabbits were randomly assigned to receive no antibiotics, vancomycin (15 mg/kg of body weight every 12 h [BID] intravenously), vancomycin plus netilmicin (2.5 mg/kg BID intramuscularly), vancomycin plus rifampin (10 mg/kg BID intramuscularly), and vancomycin plus netilmicin plus rifampin at the same routes, dosages, and schedules mentioned above. Three additional randomized groups were added later. In those groups, which were controls, netilmicin alone or in combination with vancomycin (15 mg/kg of body weight BID intravenously), was given once daily (OD) at a dose of 6 mg/kg of body weight intramuscularly for 12 days in order to achieve peak concentrations in serum greater than the MBC for the studied strain administered at an inoculum of $\simeq 5 \times 10^7$ CFU/ml. This addition was considered necessary since OD administration of aminoglycosides is considered advantageous. For intramuscular administration, the injection sites were rotated and the antibiotics given in combination were administered separately in opposite legs. Six animals in the control group of the additional experiments were sacrificed 24 h after the inoculation of *S. aureus* in order to determine the bacterial burden in vegetations at the time of initiation of therapy.

Animals surviving for the duration of experiment were sacrificed at least 15 h after the administration of the last dose of antibiotics, in order to avoid a carryover effect. The animals were sacrificed by rapid intravenous injection of sodium phenobarbital (30 mg/kg). At the time of sacrifice, aortic valvular and left ventricular vegetations were excised, weighed, homogenized (in the presence of 1 ml of sterile 0.9% NaCl), and quantitatively cultured onto blood agar plates after eight dilutions, with a 1-log inoculum difference between each dilution, in order to avoid a carryover effect. After incubation for 18 h at 35°C, the colonies of *S. aureus* growing on the agar were counted, and the result was expressed as $\log_{10}$ CFU per gram of vegetation. In calculating the mean bacterial densities in the vegetations, culture-negative vegetations were considered to contain 2 $\log_{10}$ CFU/g on the basis of the average weight of the vegetation. The same procedure described above was performed at any time that rabbits were found dead, but no longer than 8 h before postmorten examination. It should be pointed out that during the experiments, blood samples for quantitative culture were obtained from the ear artery before the administration of the first dose of antibiotics, on days 4 and 7 (for the 12-day regimen), and at the time of sacrifice.

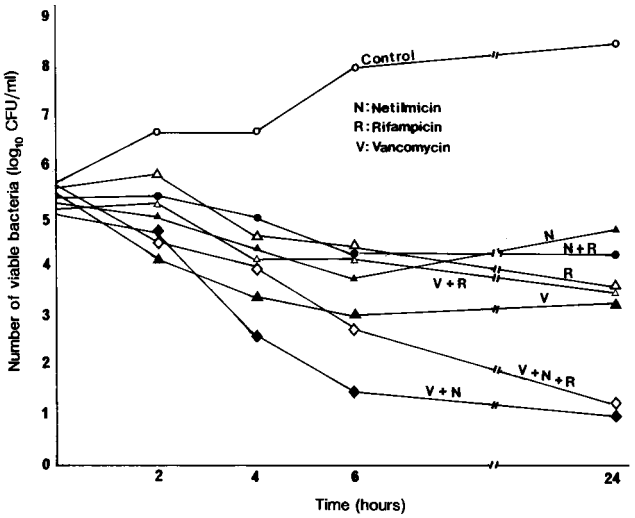

FIG. 1. Time-kill curve for the MGRSA strain by study drugs and their combinations at an inoculum of $\simeq 5 \times 10^5$ CFU/ml. The antibiotic concentrations used were 1, 4, and 4 μg of vancomycin, netilmicin, and rifampin per ml, respectively (concentrations equivalent to the MBCs of the antibiotics at inocula of $\simeq 5 \times 10^5$ CFU/ml). Data are the means of two separate experimental runs.

Antibiotic concentrations in serum. The peak and trough (only for the additional experiments) concentrations of vancomycin and netilmicin were determined on day 4 or 5 of therapy by the fluorescence polarization immunoassay (TDx system, Abbott Laboratories, Abbott Park, Ill.) (20). Samples were obtained approximately 45 to 50 min after the end of the administration; however, because of occasional technical difficulties, samples were not obtained from all animals.

SBTs. Serum samples were obtained at the time of peak antibiotic concentrations to determine the serum bactericidal titers (SBTs) in rabbit serum as described by Schlichter and MacLean (34). The SBT was defined as the highest dilution of serum that killed ≥99.9% of organisms after incubation for 18 h at 35°C.

Statistical analysis. Comparisons of frequencies of sterilization of blood cultures and vegetations were made by the Fisher exact test. Differences in bacterial vegetation counts between controls and the treatment subgroups were assessed by the Kruskal-Wallis test with pair-wise comparisons; the Mann-Whitney rank sum test was used for multiple comparisons (17). Finally, the Fisher exact test was used to compare the survival rates among the study groups.

RESULTS

In vitro susceptibility studies. The MICs/MBCs of the studied antibiotics for the MGRSA strain given at inocula of $\simeq 5 \times 10^5$ and $\simeq 5 \times 10^7$ CFU/ml were as follows: oxacillin, 64/128 and 128/256 μg/ml; gentamicin, 128/128 and 128/128 μg/ml; netilmicin, 2/4 and 4/8 μg/ml; rifampin, 1/4 and 2/8 μg/ml; and vancomycin, 1/1 and 1/1 μg/ml, respectively. After applying the aminoglycoside resistance patterns, this strain seemed to produce a bifunctional [APH(2″) + AAC(6′)] enzyme. Time-kill studies done with 4 μg of netilmicin, per ml (MBC at an inoculum of $\simeq 5 \times 10^5$ CFU/ml) demonstrated a synergistic effect at 24 h for an inoculum of $\simeq 5 \times 10^5$ CFU/ml and for the combinations of vancomycin plus netilmicin and vancomycin plus netilmicin plus rifampin (Fig. 1). In contrast, for an inoculum of $\simeq 5 \times 10^7$ CFU/ml all combinations exhibited indifferent results (data not shown). Time-kill studies done with 8 μg of netilmicin per ml (MBC at an inoculum of $\simeq 5 \times 10^7$ CFU/ml) demonstrated synergy for all combinations studied at 24 h and for an inoculum of $\simeq 5 \times 10^7$ CFU/ml (Fig. 2). Nevertheless, more than 10^5 CFU of bacteria per ml was viable

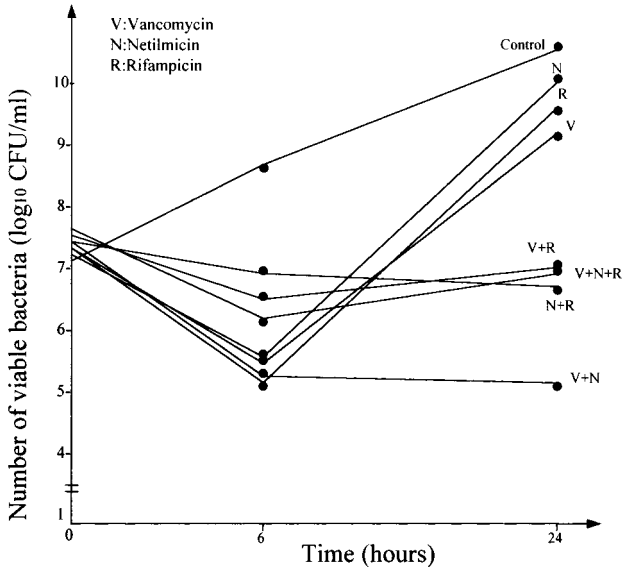

FIG. 2. Time-kill curve for the MGRSA strain by study drugs and their combinations at an inoculum of $\simeq 5 \times 10^7$ CFU/ml. The antibiotic concentrations used were 1, 8, and 8 μg of vancomycin, netilmicin, and rifampin per ml, respectively (concentrations equivalent to the MBCs of the antibiotics at inocula of $\simeq 5 \times 10^7$ CFU/ml). Data are the means of two separate experimental runs.

after exposure to these antibiotic combinations after 24 h. No increases in the MICs and the MBCs for the viable bacteria were observed.

Studies in animals. Of the 189 rabbits used in the study, 15 died within less than 24 h after placement of the catheter, 33 were excluded from further analysis because they did not meet the inclusion criteria, and 6 (in the control group) were sacrificed at 24 h postinoculation of *S. aureus*. Overall, 135 rabbits were finally evaluated for vegetation sterilization and reductions of bacterial counts in the vegetations. Table 1 presents the percentage of all animals analyzed that survived to the time of sacrifice. No statistically significant differences were observed in the survival rate or the blood culture sterilization rate among all treatment subgroups except for netilmicin versus all other treatment subgroups (Tables 1 and 2). The terminal (at day 6 or 12) blood cultures were found to be 100% sterile in the vancomycin, vancomycin plus rifampin, and the triple-combination subgroups, while they were found to be 70 to 90% sterile in the subgroups treated with the combination of vancomycin plus netilmicin (*P* was not significant) (Table 2). Cultures of all vegetations from untreated controls and the netilmicin-treated animals were positive for MGRSA. The vegetations of six control animals that were sacrificed 24 h after the inoculation of *S. aureus* contained 6.73 ± 0.46 CFU/g (mean ± standard error). As shown in Table 3, there were no significant differences among the treatment subgroups (except for netilmicin alone versus all other treatment subgroups) in the sterilization of the vegetations or a reduction in the bacterial counts in the vegetations. All treatment subgroups were more effective than the controls (except netilmicin alone for the number of sterile vegetations). The results obtained after the 12-day course of treatment were not statistically significant in comparison with the results obtained after the 6-day course. It should be pointed out that no significant MIC and MBC increases for any of the MGRSA strains obtained from vegetations after the end of treatment by any of the regimens studied were observed.

TABLE 1. Effects of different therapeutic schedules against experimental MGRSA endocarditis on survival of rabbits

Regimen[a]	Survival rate (no. of survivors/no. of animals [%])[b]	
	6 days	12 days
Controls	0/28[c]	
Vancomycin	9/11 (82)	8/11 (73)
Netilmicin (OD)	ND[d]	2/9 (22)[e]
Vancomycin + netilmicin (BID)	10/11 (91)	10/10 (100)
Vancomycin + netilmicin (OD)	ND	8/10 (80)
Vancomycin + rifampin	8/12 (67)	10/13 (85)
Vancomycin + netilmicin (BID) + rifampin	8/10 (80)	10/13 (77)

[a] OD, 6 mg/kg once daily; BID, 2.5 mg/kg every 12 h.
[b] For all correlations except those indicated, *P* was not significant.
[c] $P < 0.001$ for all treatment subgroups (for both 6- and 12-day regimens) versus controls except netilmicin (OD).
[d] ND, not done.
[e] $P = 0.027$ to $P < 0.001$ for netilmicin (OD) versus all treatment subgroups except vancomycin (*P* was not significant).

Antibiotic concentrations in serum and SBTs. Drug concentrations in serum and SBTs are given in Table 4. With minor exceptions, there were no differences in drug concentrations among the animals treated with vancomycin alone or in the combinations. All individual peak levels of vancomycin were greater than the MBC for the infecting MGRSA strain by 15- to 25-fold. Netilmicin levels slightly exceeded the MBC (at an inoculum of $\simeq 5 \times 10^5$ CFU/ml) when the dosage given was 2.5 mg/kg BID. In contrast, netilmicin levels were greater than the MBC (at an inoculum of $\simeq 5 \times 10^7$ CFU/ml) when the dosage given was 6 mg/kg OD. Rifampin levels were not measured in the present study, but it is known from previous studies that levels in serum range from 2 to 13.4 μg/ml (mean ± standard error, 5.83 ± 0.84 μg/ml) after rabbits are given a dosage of 10 mg/kg BID intramuscularly (2). Peak SBTs for animals treated with vancomycin were higher (but not at a significant level) than those for animals treated with the combinations. Additionally, peak SBTs higher than 1:8 were not correlated with a better therapeutic outcome.

DISCUSSION

S. aureus is responsible for 25 to 35% of cases of native valve left-sided endocarditis and for up to 70% of cases of right-sided infection (23). In recent years, MRSA strains have increasingly been isolated, and they are incriminated in both community-acquired and severe nosocomial infections, including infective endocarditis (5, 12, 29). Even though vancomycin, in comparison with the antistaphylococcal penicillins, is considered to exhibit slower killing rates in vitro against staphylococci (24, 25), it is the drug of choice for the treatment of infections caused by MRSA, as well as for the treatment of patients with a history of anaphylaxis to β-lactams (8, 24, 25). However, the clinical efficacy of monotherapy with vancomycin has been questioned in several studies. Small and Chambers (35) and Geraci and Wilson (16), after reviewing 13 patients (11 with right-sided *S. aureus* endocarditis) and 12 patients (all with left-sided *S. aureus* endocarditis), respectively, reported 38 and 42% failure rates, respectively. On the contrary, the clinical failure of β-lactam antibiotics in more than 300 cases of *S. aureus* endocarditis in drug users compiled from 11 published series was less than 2% (35). In addition, the experience of Levine et al. (25) suggests that *S. aureus* bacteremia is terminated less rapidly after treatment with vancomycin than after treatment with nafcillin. Therefore, it is of great practical

TABLE 2. Effects of different therapeutic schedules on blood culture sterilization in experimental MGRSA endocarditis

Regimen[a]	No. of sterile blood cultures/no. of animals alive (%) on:				
	6-day regimen		12-day regimen		
	Day 4	Day 7[b]	Day 4	Day 7	Day 13[b]
Controls[c]	0/5 (0)				
Vancomycin	9/9 (100)	8/8 (100)	10/11 (91)	10/10 (100)	8/8 (100)
Netilmicin (OD)	ND[d]	ND	1/5 (20)	1/2 (50)	1/2 (50)
Vancomycin + netilmicin (BID)	9/11 (82)	8/10 (80)	8/10 (80)	9/10 (90)	7/10 (70)
Vancomycin + netilmicin (OD)	ND	ND	7/10 (70)	7/8 (87)	6/8 (75)
Vancomycin + rifampin	9/10 (90)	7/7 (100)	10/11 (91)	9/9 (100)	10/10 (100)
Vancomycin + netilmicin (OD) + rifampin	8/9 (89)	8/8 (100)	9/11 (82)	10/10 (100)	10/10 (100)

[a] OD, 6 mg/kg once daily; BID, 2.5 mg/kg every 12 h.
[b] Blood for culture was drawn just before the sacrifice.
[c] No control animals were alive at day 7.
[d] ND, not done.

interest to clarify whether the combination of vancomycin with another antibiotic might produce better therapeutic results. The addition of gentamicin to vancomycin in humans, while it does not improve cure rates, if given only for the first 3 to 5 days of therapy, has resulted in a more rapid clearing of bacteremia, while it avoids the nephrotoxicity associated with prolonged courses of aminoglycoside therapy. Rifampin, on the other hand, is an attractive agent for use in combination with vancomycin because (i) it is highly bactericidal in vitro against *S. aureus* (36), (ii) it possesses optimal pharmacokinetics in most body fluid compartments (1), and (iii) it penetrates into leukocytes, acting bactericidally in the cell (27) and being advantageous in cases of myocardial or metastatic abscesses complicating infective endocarditis (25, 38). However, the in vitro findings are contradictory because all kinds of interactions with vancomycin have been described (3, 19, 36, 39). In vivo studies both in animals and in humans aiming to clarify the efficacy of the combination of vancomycin plus rifampin against serious MRSA infections, including endocarditis, also yielded conflicting results (2, 4, 23, 25, 38).

On the basis of the contradictory information reported elsewhere, the present experimental study was designed in an effort to elucidate the role of netilmicin against MGRSA after considering that MGRSA isolates are often susceptible to netilmicin (16a); that, compared with gentamicin, netilmicin has better pharmacokinetic properties within the vegetation (13); and that netilmicin is less nephrotoxic (11, 22, 26, 31). The present study was also designed to redefine the role of the addition of rifampin to vancomycin or to the combination of vancomycin plus netilmicin and to investigate the influence of the duration of therapy on different therapeutic regimens.

The dosage regimens used in the present study were chosen because they were effective in the treatment of experimental endocarditis in previous studies (2, 7). Netilmicin at a dose of 2.5 mg/kg given intramuscularly achieved mean peak concentrations in the sera of rabbits (4.2 μg/ml) quite lower than those in the sera of humans (6 μg/ml) after administration of a dose of 2 mg/kg given intramuscularly (6 μg/ml) (30).

In the present study it was found that, with the exception of the netilmicin monotherapy schedule, all applied therapeutic regimens, namely, vancomycin alone, vancomycin plus netilmicin, vancomycin plus rifampin, and the triple combination, were very effective at eliminating the bacteremia, improving the survival rates, increasing the number of sterile vegetations, and improving the persisting bacterial counts in the vegetations, especially whenever treatment was extended to 12 days. The unusually high bacterial load within the vegetations of untreated animals emphasizes the value of the observed results. An exception, however, was the decreased, but not statistically significant, efficacy of the combination of vancomycin

TABLE 3. Effects of different therapeutic schedules on vegetation sterilization and vegetation bacterial counts in experimental MGRSA endocarditis[a]

Regimen[b]	No. of sterile vegetations/total no. of vegetations (%)		Bacterial counts in vegetation (mean ± SE $\log_{10}$ CFU/g)	
	6 days	12 days	6 days	12 days
Control	0/28 (0)[c]		11.28 ± 0.50[d]	
Vancomycin	6/10 (60)	9/11 (82)	5.63 ± 1.61	3.03 ± 0.73
Netilmicin (OD)	ND[e]	0/9 (0)[f]	ND	9.06 ± 0.39[g]
Vancomycin + netilmicin (BID)	8/11 (73)	5/10 (50)	5.10 ± 1.60	5.65 ± 1.70
Vancomycin + netilmicin (OD)	ND	5/10 (50)	ND	4.11 ± 0.83
Vancomycin + rifampin	4/11 (36)	9/12 (75)	5.27 ± 0.99	3.77 ± 1.01
Vancomycin + netilmicin (BID) + rifampin	7/10 (70)	11/13 (85)	4.45 ± 1.31	3.12 ± 0.77

[a] Unless indicated otherwise P for all correlations was not significant.
[b] OD, 6 mg/kg once daily; BID, 2.5 mg/kg every 12 h.
[c] $P < 0.001$ for all treatment subgroups (for both 6- and 12-day regimens) versus controls except for vancomycin plus rifampin for the 6-day regimen ($P < 0.01$) and netilmicin (OD) (P was not significant).
[d] $P < 0.001$ for all treatment subgroups (for both 6- and 12-day regimens) versus controls except for vancomycin for the 6 day regimen ($P < 0.01$); vancomycin plus netilmicin (BID) for the 12-day regimen ($P < 0.01$), and netilmicin (OD) ($P < 0.05$).
[e] ND, not done.
[f] $P < 0.05$ to 0.001 for netilmicin (OD) versus all other treatment subgroups.
[g] $P < 0.001$ for netilmicin (OD) versus all other treatment subgroups except vancomycin plus netilmicin (BID).

TABLE 4. Levels in serum and peak bactericidal titers in MGRSA endocarditis

Regimen[a]	Concn (μg/ml)[b]		Median SBT (range)
	Vancomycin	Netilmicin	
Vancomycin (n = 13)	21.8 ± 2.8 (25.6–19.2)		1/8 (<1/4–1/16)
Netilmicin (OD) (n = 5)		12.4 ± 2.1 (15.0–9.3)[c]	ND[d]
Vancomycin + netilmicin (BID) (n = 10)	20.1 ± 4.3 (26.4–15.2)	4.2 ± 0.8 (5.6–3.3)	1/4 (<1/4–1/8)
Vancomycin + netilmicin (OD) (n = 7)	26.0 ± 6.2 (37.5–18.7)[e]	13.0 ± 2.9 (17.6–9.1)[f]	ND
Vancomycin + rifampin (n = 8)	20.9 ± 4.2 (27.7–15.6)		<1/4 (<1/4–1/16)
Vancomycin + netilmicin (BID) + rifampicin (n = 9)	23.5 ± 6.5 (36.9–17.4)	4.6 ± 0.7 (6.1–4.1)	<1/4 (<1/4–1/8)

[a] n, number of samples tested; OD, 6 mg/kg once daily; BID, 2.5 mg/kg every 12 h.
[b] Values are means ± standard deviations (ranges) and represent the peak concentrations.
[c] The trough levels were <0.1 μg/ml.
[d] ND, not done.
[e] The trough levels were 5.4 ± 1.4 μg/ml (range, 7.3 to 3.9 μg/ml).
[f] The trough levels were 0.15 ± 0.07 μg/ml (range, 0.1 to 0.24 μg/ml).

plus netilmicin (BID) in the 12-day treatment schedule in comparison with that in the 6-day schedule. The latter event was observed, even though the levels of the antibiotics in serum and the SBTs were similar in both subgroups and the emergence of resistance did not occur, while the survival rate was one of the best among the different subgroups. Regarding the efficacy of rifampin, despite the synergistic results reported by Bayer and Lam (2), it is clear from the present study that the addition of rifampin to vancomycin does not improve the efficacy of the latter drug.

The apparent discrepancy between the in vitro and the in vivo results merits further discussion. The time-kill studies demonstrated either bactericidal synergy with combinations containing netilmicin at 4 μg/ml when they were tested against inocula of 5×10^5 CFU/ml or nonbactericidal synergy with combinations containing netilmicin at concentrations of 8 μg/ml when they were tested against inocula of 5×10^7 CFU/ml. In the latter case, the combination of vancomycin plus netilmicin was synergistic but not bactericidal, since more than 10^5 CFU of the MGRSA strain per ml was viable after 24 h of incubation. Taking into account the fact that the bacterial burden in vegetations at the time of the initiation of therapy was as high as 10^7 CFU/g, increasing to the huge number of 10^{11} CFU/g 3 days later (mean number of CFU per gram and mean survival time of the untreated animals, respectively) and the results of the time-kill curve studies with inocula of 5×10^7 CFU/ml, someone could easily conclude that the in vitro results predicted to some degree the in vivo results regarding the combination of netilmicin plus vancomycin. In contrast, the time-kill curve study did not predict the good in vivo results obtained with vancomycin. The probable explanation for this discrepancy is that the concentrations of vancomycin in serum were more than 20 (peak) or 5 (trough) times greater than the concentrations of vancomycin (equal to the MBC) used in the in vitro studies. The fact that therapy was started 24 h after bacterial challenge, when the bacterial burden in vegetations was $\simeq 10^7$ CFU/ml, an inoculum for which the MBC of vancomycin was only 1 μg/ml in vitro, might have facilitated the effect of vancomycin.

To our knowledge this is the first experimental study showing that prolonged treatment with vancomycin alone has excellent activity against MGRSA aortic valve endocarditis. Our results are in agreement with those of Levine et al. (25) in human native valve MRSA left- or right-sided endocarditis, who reported an 86% cure rate with vancomycin used as monotherapy. Although the latter investigators included mostly patients with tricuspid valve infection, for which the prognosis is much better, they estimated that the median duration of bacteremia was delayed by 9 and 7 days for left- and right-sided endocarditis, respectively. In the present experimental study, however, no significant differences in blood culture sterilization rates after 3, 6, or 12 days of therapy among the four therapy subgroups were observed, with almost 100% sterile blood cultures observed after only 3 days of therapy. The only exceptions were the vancomycin plus netilmicin subgroups, in which the blood sterilization rate was 70 to 80% (P was not significant) on the day of sacrifice.

The prognostic value of SBT regarding the therapeutic outcome has also been reported to be controversial (6, 10). In a recent study (37), peak SBTs equal to or greater than 1:64 were correlated with a better outcome. In contrast to those findings, the SBTs obtained in the present study were in agreement with the results of clinical studies, in which SBTs equal to or greater than 1:8 did not necessarily correlate with a better outcome (6, 10, 21). On the other hand, higher and lower peak SBTs were equally distributed among the different subgroups in the present study, not permitting certain conclusions to be made. Although lower SBTs were obtained whenever rifampin was administered, the final outcome was not influenced by rifampin.

In conclusion, the results of the present experimental study indicate that the administration of vancomycin as monotherapy is effective, and the addition of rifampin is not necessary, as also indicated by Levine et al. (25), in a study with humans, while coadministration of netilmicin (BID or OD), does not offer any advantage. The findings reported here merit further evaluation in humans, particularly in cases of prosthetic valve endocarditis.

ACKNOWLEDGMENTS

We thank G. Petrikkos and I. Galani for assistance with aminoglycoside resistance pattern analysis, M. Grammatikou for technical work, and K. Antoniou for secretarial assistance.

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