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# Pharmacodynamics of Once- or Twice-Daily Levofloxacin versus Vancomycin, with or without Rifampin, against *Staphylococcus aureus* in an In Vitro Model with Infected Platelet-Fibrin Clots

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**We compared the pharmacodynamic activities of levofloxacin versus vancomycin, with or without rifampin, in an in vitro model with infected platelet-fibrin clots simulating vegetations. Infected platelet-fibrin clots were prepared with human cryoprecipitate, human platelets, calcium, thrombin, and ~$10^9$ CFU of organisms (MSSA 1199 and MRSA 494) per g and then were suspended via monofilament line into the in vitro model containing Mueller-Hinton growth medium. Antibiotics were administered by bolus injection into the model to simulate human pharmacokinetics; the regimens simulated included levofloxacin at dosages of 800 mg every 24 h (q24h) and 400 mg q12h, vancomycin at 1 g q12h, and rifampin at 600 mg q24h. Each model was run in duplicate over a 72-h period. Infected platelet-fibrin clots were removed in duplicate from each model, weighed, homogenized, serially diluted with sterile 0.9% saline, and plated on tryptic soy agar plates and plates containing antibiotics at 3, 6, and 12 times the MIC to evaluate the emergence of resistance. Time-kill curves were constructed by plotting the inoculum size versus time. Residual inoculum at 72 h was used to compare regimens. All levofloxacin regimens were significantly better than vancomycin monotherapy against both isolates ($P < 0.002$). Against MSSA 1199, levofloxacin q24h was significantly better than all other regimens, including levofloxacin q12h ($P < 0.002$); however, no difference between the levofloxacin monotherapy and combination therapy (with rifampin) regimens against MRSA 494 was seen. Killing activity for levofloxacin appeared to correlate better with the peak/MIC ratio than with the area under the curve/MIC ratio. The addition of rifampin significantly enhanced the activity of vancomycin but had little effect upon the activity of levofloxacin. For MRSA 494, vancomycin plus rifampin resulted in the greatest killing ($P < 0.05$). Development of resistance was not detected with any regimen. Levofloxacin may be a useful therapeutic alternative in the treatment of staphylococcal endocarditis, and further study with animal models of endocarditis or clinical trials are warranted.**

In spite of therapeutic advances made over the past decade, endocarditis remains a difficult infection to manage. Reasons for this include the large inoculum of organisms present, poor drug penetration into vegetations, and the lack of an effective immune response due to the inability of leukocytes to reach the sequestered site that the vegetation provides (3). *Staphylococcus aureus* is one of the most common causative organisms of native valve endocarditis in the intravenous drug user population. First-line therapy consists of semisynthetic β-lactamase-stable penicillins, such as nafcillin or oxacillin, with or without an aminoglycoside, or vancomycin in cases of methicillin resistance or penicillin intolerance. Unfortunately, the incidence of infection due to methicillin-resistant isolates is increasing, resulting in increased reliance upon vancomycin, since no other universally effective therapeutic agent exists. With the advent of vancomycin-resistant enterococci and the potential for spread of resistance genes and subsequent development of glycopeptide-resistant staphylococci, there is a need for alternatives to vancomycin for the treatment of serious staphylococcal infections.

Various fluoroquinolones have been studied with animal models of endocarditis and have been found to be effective (11–13). Levofloxacin, the L-isomer of ofloxacin, possesses greater activity than the parent drug against gram-negative and gram-positive organisms and is more active than ciprofloxacin against *S. aureus* (8). Similarly to other agents in the same class, levofloxacin has a postantibiotic effect against most bacteria and exhibits concentration-dependent killing. On the basis of favorable pharmacokinetic and pharmacodynamic properties, it appears that levofloxacin may have potential in the treatment of *S. aureus* endocarditis.

The addition of rifampin as adjunctive therapy in treatment of staphylococcal endocarditis is controversial. Numerous investigators have studied the combination of rifampin with ciprofloxacin and have found conflicting results ranging from synergism to frank antagonism, similar to data reported for vancomycin (9, 10, 12, 14). It has also been reported that addition of rifampin prevented emergence of resistance to ciprofloxacin (12, 14).

The objectives of this investigation were to compare the bactericidal activities of levofloxacin administered as either a single daily dose or the equivalent dose administered twice daily, alone or in combination with rifampin, versus vancomy-

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TABLE 1. Pharmacokinetics in the in vitro model[a]

| Drug and regimen | Concn (μg/ml) | | $t_{1/2}$[b] (h) | Peak[c]/MIC | | Trough/MIC | | AUC/MIC | |
|---|---|---|---|---|---|---|---|---|---|
| | Peak (0.5 h) | Trough | | MSSA 1199 | MRSA 494 | MSSA 1199 | MRSA 494 | MSSA 1199 | MRSA 494 |
| Vancomycin | 31.4 (1.90) | 7.8 (1.12) | 5.5 (0.36) | 43.6 (1.4) | 87.2 (2.9) | 10.1 (1.5) | 20.1 (3.1) | 452.6 (14.5) | 905.0 (29.0) |
| Levofloxacin | | | | | | | | | |
| q24h | 10.0 (0.65) | 0.6 (0.06) | 5.8 (0.24) | 58.3 (3.6) | 26.6 (0.61) | 3.0 (0.03) | 1.4 (0.15) | 366.3 (19.4) | 178.5 (9.4) |
| q12h | 4.7 (0.18) | 1.2 (0.11) | 5.8 (0.24) | 27.6 (0.75) | 12.9 (0.30) | 6.5 (0.58) | 3.2 (0.28) | 357.9 (1.5) | 174.3 (0.71) |
| Rifampin | 8.63 (1.5) | <0.06 | 2.69 (0.06) | 2,597.4 (487.2) | 2,596.4 (487.2) | | | | |

[a] All values are means, with standard deviations given in parentheses.
[b] $t_{1/2}$, half-life.
[c] Maximum concentration of drug in the model extrapolated to time zero.

cin administered as monotherapy or in combination with rifampin, against methicillin-sensitive and -resistant *S. aureus* (MSSA and MRSA, respectively) strains in an in vitro model with infected platelet-fibrin clots simulating vegetations.

## MATERIALS AND METHODS

**Organisms.** Two clinical strains of *S. aureus*, one methicillin sensitive (MSSA 1199) and one methicillin resistant (MRSA 494), which were isolated from patients with endocarditis at Detroit Receiving Hospital were used in the study.

**Antibiotics.** Levofloxacin for injection (lot N-8018) and analytical powder (lot N-8017) were supplied by R. W. Johnson Pharmaceutical Research Institute, Raritan, N.J. Rifampin for injection (lot 026A; Marion Merrell Dow) and analytical powder (lot 81H331; Sigma Chemical Co.) and vancomycin for injection (lot 140256; Lyphomed) and analytical powder (112H075025; Sigma) were obtained commercially.

**Susceptibility testing.** MICs and MBCs were determined for each drug by using analytical powder and utilizing broth microdilution techniques according to National Committee for Clinical Laboratory Standards guidelines with an inoculum of $5 \times 10^5$ CFU/ml (19). The presence or lack of an inoculum effect was determined by repeating MIC and MBC determinations with a larger inoculum ($5 \times 10^8$ CFU/ml). Synergy testing was performed by the checkerboard method (synergy, fractional inhibitory concentration index of ≤0.5; additivity, >0.5 to 1.0; indifference, >1.0 to <4.0; antagonism, ≥4.0) (7).

**Medium.** Mueller-Hinton broth (Difco, Detroit, Mich.) supplemented with 25 mg of calcium per ml and 12.5 mg of magnesium per ml (SMHB) was utilized in all experiments.

**Preparation of infected platelet-fibrin clots.** Organism stock was prepared by inoculating test tubes containing 10 ml of SMHB with approximately three to five colonies harvested from a fresh overnight tryptic soy agar (TSA) (Difco) plate. The test tubes were placed in a rotator apparatus and housed in an incubator at 35°C overnight. The tubes were centrifuged and the supernatant was decanted, leaving a concentrated organism pellet in the bottom of each tube (inoculum size, $\sim 10^{10}$ CFU/ml). Organism pellets were combined and resuspended in a small amount of SMHB in order to achieve a final inoculum of $10^9$ CFU/0.1 ml.

Infected platelet-fibrin clots were prepared from a modified fibrin glue recipe (22, 24) by mixing 0.9 ml of human cryoprecipitate (American Red Cross), 0.1 ml of organism suspension (final inoculum, $10^9$ CFU/g), 0.05 ml of platelet suspension (diluted in 0.9% NaCl to provide ~250,000 platelets per fibrin clot), and 0.05 ml of aprotinin solution (2,000 Kallikrein inhibitory units/ml; Sigma) in sterile, siliconized 1.5-ml Eppendorf tubes. A sterile, monofilament line was inserted into each clot, and 0.1 ml of bovine thrombin solution (1,000 U/ml) (Thrombostat; Parke-Davis) reconstituted with calcium (500 mM/liter) was added. The clots were allowed to gel completely and then were removed with sterile 22-gauge needles and immediately suspended into the in vitro model.

**In vitro model.** An in vitro model which has been previously described was utilized (18). Infected platelet-fibrin clots were suspended via monofilament lines into the model through ports which were then sealed with rubber stoppers. Antibiotics were administered by bolus injection into the model to achieve peak concentrations similar to those achieved in humans receiving the parenteral dosage regimens listed below. Fresh SMHB was pumped into each model while antibiotic-containing medium was pumped out at an equal rate to achieve drug elimination. For combination therapy experiments, the elimination rate was set for the drug with the shortest half-life, and the drug with the longest half-life was supplemented as previously described (1). The elimination half-lives targeted were ~6 h for levofloxacin and vancomycin and 3 h for rifampin. The drug regimens and peak concentrations simulated were as follows: vancomycin at 1 g every 12 h (q12h) (30 to 40 μg/ml), levofloxacin at 400 mg q12h (5 μg/ml) and at 800 mg q24h (10 μg/ml), and rifampin at 600 mg q24h (8 to 10 μg/ml). Magnetic stir bars placed in the model ensured thorough mixing of drugs and growth medium and aided in temperature maintenance. The entire apparatus was maintained in a water bath at 37°C.

**Pharmacodynamic and pharmacokinetic analyses.** At 0, 12, 24, 48, and 72 h, fibrin clots were removed in duplicate from each model, placed in sterile petri dishes, weighed, and then transferred aseptically to sterile 3-ml tubes filled one-third full with 3-mm-diameter glass beads. The clots were homogenized by adding 0.5 ml of 1.25% trypsin solution and then placing them in a mini bead-beater (BioSpec Products, Bartlesville, Okla.) on high speed for 3 to 5 min. The homogenate was then serially diluted with cold sterile 0.9% sodium chloride, plated on TSA plates, and incubated at 35°C for 18 to 24 h, at which time the colonies were counted and the inoculum size was determined. Samples were also plated on TSA plates containing antibiotics at 3, 6, and 12 times the MIC and incubated for 48 h for detection of resistant subpopulations. At times when bacterial counts were expected to be unreliable as indicated by results from antibiotic carryover experiments, 100-μl samples were diluted in 10 ml of 0.9% NaCl and filtered with 0.45 μm filters. The filters were then placed aseptically on TSA plates and incubated for 18 to 24 h, at which time the colonies were counted and the inoculum size was determined. The limit of detection of the filtration method was determined to be 100 CFU/ml for staphylococci ($n = 20$; between-day coefficient of variation, 3.5%). Time-kill curves were constructed by plotting the inoculum size ($\log_{10}$ CFU per gram) versus time. The time to 99.9% killing, if achieved, was determined by visual inspection. At 0.5, 1, 4, 8, 12, 24, 36, 48, and 72 h, 0.5-ml samples were removed from the model for pharmacokinetic analysis. The samples were frozen at −70°C until assay. The area under the concentration-time curve (AUC) was calculated for the central compartment from 0 to 24 h by the trapezoid method utilizing the LAGRAN program, version 2.1 (20). The maximum concentration of drug in the model for each drug dosage regimen was extrapolated to time zero from the concentration-versus-time plot by linear regression. Peak and trough concentration/MIC ratios as well as the 24-h AUC/MIC ratio for each antibiotic regimen against both isolates were evaluated to determine if a relationship existed between these parameters and bacterial killing. Vancomycin concentrations were determined by a fluorescence polarization immunoassay (TDx; Abbott Laboratories, Irving, Tex.), which has a sensitivity of 0.6 μg/ml and between-day coefficients of variation of <6% (coefficient of determination [$r^2 = 0.99$) (21). Levofloxacin and rifampin concentrations were measured by microbioassays using *Klebsiella pneumoniae* ATCC 10031 and *Micrococcus luteus* ATCC 9341, respectively. The assay limits of detection are 0.3 μg/ml for levofloxacin and 0.06 μg/ml for rifampin, with between-day coefficients of variation of <2% and 7%, respectively. $r^2$ for both assays was ≥0.96 over the concentration range of 0.3 to 2.7 μg/ml for levofloxacin and 0.06 to 1.0 μg/ml for rifampin.

**Statistical analysis.** Analysis of variance with Tukey's test for multiple comparisons was used to compare differences in the numbers of residual organisms at 72 h in order to discern differences between drug regimens. A $P$ value of <0.05 was considered significant.

## RESULTS

**Susceptibility studies.** The MICs and MBCs, respectively, of vancomycin, levofloxacin, and rifampin for each isolate were as follows: MSSA 1199, 0.78 and 1.56, 0.19 and 0.39, and 0.0039 and 0.25; MRSA 494, 0.39 and 0.78, 0.39 and 0.39, and 0.0039 and 0.25. No inoculum effect was observed with levofloxacin, but the MICs and MBCs of vancomycin increased slightly at the higher inoculum to 3.13 and 12.5, respectively, for both strains. In synergy studies using the checkerboard technique, the combination of rifampin with either vancomycin or levofloxacin was indifferent with both bacterial isolates (fractional inhibitory concentration index, 2 to 4).

**Pharmacodynamics and pharmacokinetics.** The pharmacodynamic and pharmacokinetic parameters for the model experiments are summarized in Table 1. Drug concentrations in the model remained above the MICs for the organisms for the

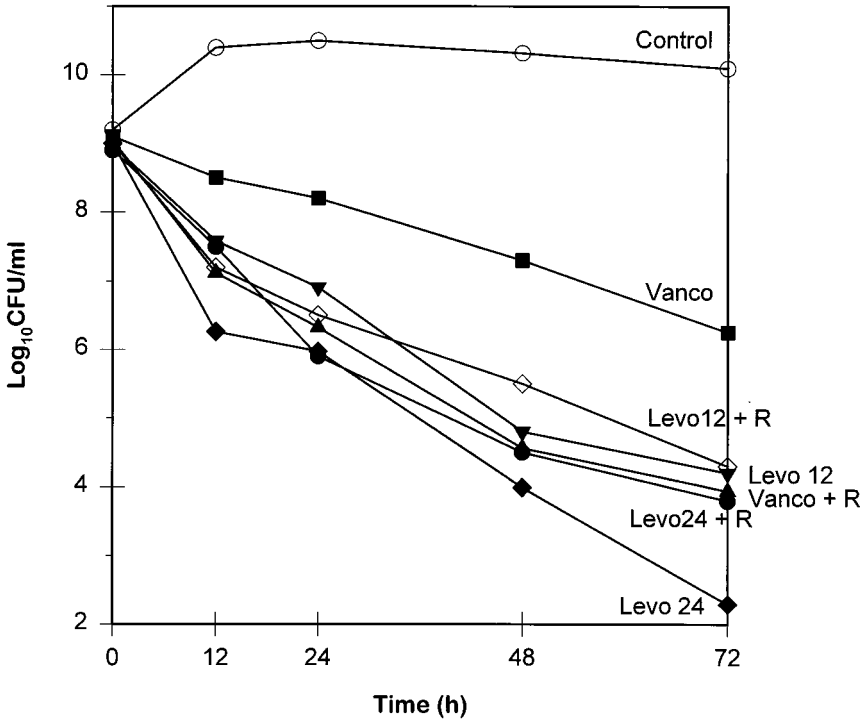


FIG. 1. Time-kill curves for MSSA 1199 in the in vitro model. Vanco, vancomycin; R, rifampin; Levo 12 and 24, levofloxacin at 400 mg q12h and 800 mg q24h, respectively.

entire dosing interval with both vancomycin and levofloxacin regimens. As expected, the AUC for the levofloxacin regimen of 800 mg q24h was similar to that achieved by levofloxacin at 400 mg q12h, while peak concentrations achieved by each regimen were significantly different. Therefore, the peak/MIC ratios achieved by the once-daily regimen were twice as high as those achieved with the twice-daily regimen, and the AUC/MIC ratios were approximately equivalent.

Figures 1 and 2 depict time-kill curves for the two isolates of *S. aureus*. All regimens, with the exception of vancomycin monotherapy, achieved 99.9% killing within 48 h. The numbers of residual organisms at 72 h for all drug regimens are listed in Table 2. All levofloxacin regimens were significantly better than vancomycin monotherapy against both isolates ($P < 0.0002$). Levofloxacin q24h was more effective than all other regimens, including levofloxacin q12h, against MSSA 1199 ($P < 0.0002$); however, no difference was seen between the levofloxacin monotherapy and combination therapy regimens against MRSA 494. The addition of rifampin significantly enhanced the activity of vancomycin against both isolates but had little effect upon the activity of levofloxacin. Against MRSA 494, vancomycin plus rifampin resulted in the greatest killing ($P < 0.05$). Development of resistance was not detected with any regimen.

## DISCUSSION

Vancomycin has traditionally been used in the treatment of endocarditis due to methicillin-resistant strains of staphylococci or other gram-positive organisms in patients with penicillin allergy. However, it is increasingly recognized that patients with deep-seated infections tend to respond more slowly to vancomycin than to other antibiotics to which the infecting organism is susceptible (2, 15, 23). In our investigation, vancomycin monotherapy was the only drug regimen which did not achieve a decrease in inoculum of 3 log units over the 72-h period. The addition of rifampin resulted in significantly improved killing activity; however, synergy between rifampin and vancomycin against *S. aureus* has been shown to be strain dependent and difficult to predict by standard in vitro test methods (15, 25). Indeed, as demonstrated in this experiment, the use of the checkerboard method was not predictive of the synergy observed in the in vitro model. These results are similar to our previous experience comparing the two methods for determination of synergy (14).

It has been postulated that differences in the extent of penetration and pattern of distribution of various antibiotics may influence the ability of an antimicrobial agent to sterilize vegetations. In an elegant series of experiments, Cremieux and colleagues utilized radiolabeled drugs to study penetration into endocardial vegetations in a rabbit model of streptococcal endocarditis (3–5, 17). Interestingly, concentrations of fluoroquinolones, tobramycin, and amoxicillin-clavulanate were found to be homogeneous throughout, indicating that these agents penetrate the vegetation rapidly and completely (3–5, 17). Penicillin exhibited a concentration gradient which was highest at the periphery but decreased substantially toward the core, while an investigational glycopeptide, teicoplanin, was detected only at the periphery of the vegetations. The authors suggested that molecular weight or electric charge may hinder diffusion into the fibrin matrix. If this is true, then vancomycin may act in similarly to teicoplanin. Inability of the drug to reach the site of infection, then, could be a contributing factor which results in the prolonged bacteremia and suboptimal outcome reported for staphylococcal endocarditis patients treated with vancomycin (2, 15, 23).

The efficacy or failure of an antimicrobial regimen in the treatment of bacterial endocarditis may be dependent upon a

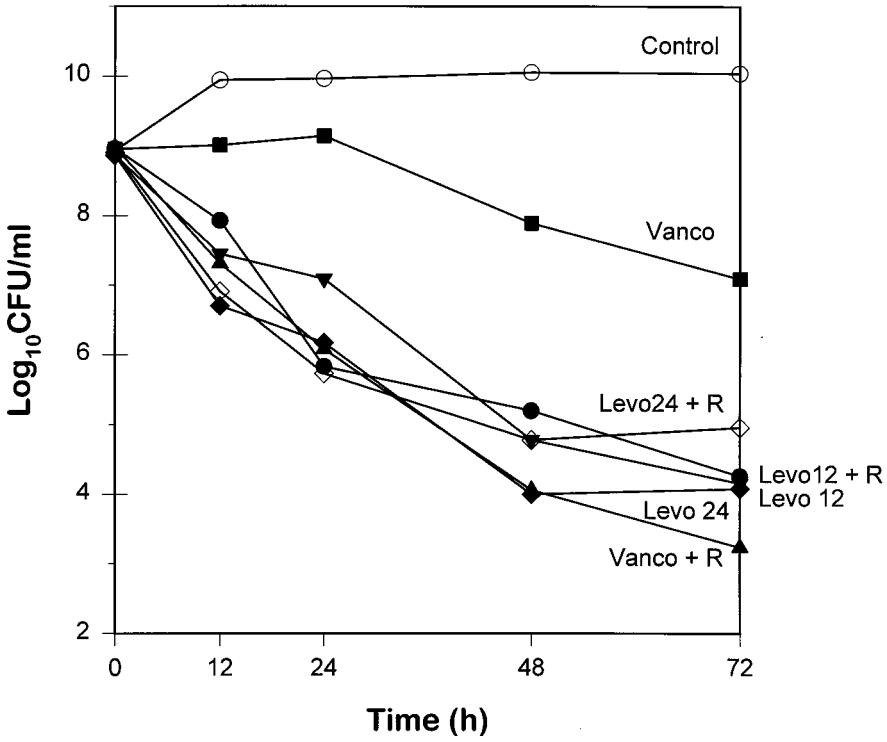


FIG. 2. Time-kill curves for MRSA 494 in the in vitro model. Vanco, vancomycin; R, rifampin; Levo 12 and 24, levofloxacin at 400 mg q12h and 800 mg q24h, respectively.

myriad of other drug-related factors, including the killing rate of the antibiotic, existence of a postantibiotic effect, metabolic state of the bacteria, effect of bacterial inoculum, and mutation frequency of the organism (3). In these respects, fluoroquinolones possess several advantages over glycopeptides, such as more-complete penetration into the vegetations, rapid bactericidal activity against both logarithmic-phase and stationary-phase organisms, concentration-dependent killing, and a lack of an inoculum effect. In addition, a secondary mechanism of action against nondividing organisms has been described for ofloxacin and staphylococci that was not found with ciprofloxacin (16). A remaining concern is the potential for resistance. Although emergence of resistance to ciprofloxacin is commonly seen with staphylococci, resistance to ofloxacin or levofloxacin has rarely been described, even in controlled studies using the same bacterial isolates. In an animal model of staphylococcal endocarditis, resistance to ciprofloxacin emerged in 12.5 to 82% of animals studied, while resistance to ofloxacin was not detected (11–13). In an in vitro infection model, Kang et al. reported results which were similar in that resistance to ciprofloxacin but not to ofloxacin or levofloxacin emerged (14). Ciprofloxacin resistance was suppressed by the addition of rifampin. The reason for differences in the incidence of resistance with these fluoroquinolones is not known. These findings are significant and require further study.

In our investigation, levofloxacin was superior to vancomycin monotherapy against strains of MSSA and MRSA in an in vitro model of endocarditis. We found that the once-daily regimen was more rapidly bactericidal and had either the same efficacy as or greater efficacy than the twice-daily regimen. Killing activity, at least initially, appeared to correlate better with the peak/MIC ratio than with the AUC/MIC ratio. These results were not surprising, since quinolones such as levofloxacin show concentration-dependent killing. The once-daily levofloxacin regimen achieved peak/MIC ratios of >25/1 against both isolates. We have observed a similar relationship for levofloxacin, ofloxacin, and ciprofloxacin in a previous in vitro infection model against the same *S. aureus* isolates (14). Drusano et al. also demonstrated that peak/MIC ratios of >20/1 for the fluoroquinolone lomefloxacin predicted successful outcomes better than AUC/MIC ratios in a neutropenic rat infection model (6). The addition of rifampin to the fluoroquinolone regimens did not enhance killing; in fact, there appeared to be mild antagonism of levofloxacin activity at 72 h. Development of resistance to levofloxacin was also not observed, which was similar to our previous experience with levofloxacin and ofloxacin (13, 14). Vancomycin's bactericidal activity was predictably slower than that of levofloxacin, despite the

TABLE 2. Residual organisms at 72 h

| Regimen | Mean CFU/g (SD)[a] | |
|---|---|---|
| | MSSA 1199 | MRSA 494 |
| None (growth control) | 10.07 (0.03) | 10.04 (0.19) |
| Vancomycin | | |
| Alone | 6.25 (0.12) | 7.09 (0.27) |
| With rifampin | 3.94* (0.10) | 3.24*[b] (0.21) |
| Levofloxacin q12h | | |
| Alone | 4.17* (0.08) | 4.16* (0.07) |
| With rifampin | 4.29* (0.04) | 4.26* (0.24) |
| Levofloxacin q24h | | |
| Alone | 2.29** (0.39) | 4.08* (0.06) |
| With rifampin | 3.83* (0.06) | 4.96* (0.97) |

[a] *, $P < 0.0002$ versus vancomycin; **, $P < 0.0002$ versus all regimens.
[b] $P < 0.05$ versus all regimens.

achievement of trough/MIC ratios of >10/1 for each isolate. The addition of rifampin to vancomycin dosing regimens has been controversial (15, 25). In the present study, bactericidal activity was significantly improved by the addition of rifampin. Since both antagonism and synergy have been observed in vitro when rifampin has been added to vancomycin for use against *S. aureus*, our results may be strain specific and therefore may not be predictable with other isolates. Our model does possess limitations, including the lack of a host defense system and the absence of other conditions that an endocardial vegetation would be exposed to in vivo (e.g., turbulence created by the flow of blood over the damaged valve, etc.). In addition, the duration of our experiments (72 h) is significantly less time than is necessary to sterilize vegetations in human subjects. However, we feel that this model may provide an initial screening tool for antibiotics with potential use in the treatment of endocarditis. Various dosing regimens, including combination therapies, can be studied in vitro, with the data generated from these experiments utilized to develop better protocols for study first in animal models and finally in humans. On the basis of our results, it appears that levofloxacin may have potential as an alternative in the treatment of both MSSA and MRSA endocarditis. Further study evaluating the efficacy of once-daily levofloxacin regimens is warranted.

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