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In Vivo Verification of In Vitro Model of Antibiotic Treatment of Device-Related Infection

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Device-related infections are difficult to treat with antibiotics alone. Standard susceptibility tests do not correlate with treatment success. Therefore, the utility of a pharmacokinetic in vitro model has been evaluated in comparison with the tissue-cage infection model in guinea pigs. The bactericidal activity of 28 treatment regimens has been studied by using three different test strains. In vitro efficacy was defined as reduction in the number of suspended or adherent bacteria, and in vivo efficacy was defined as reduction in the number of bacteria in tissue-cage fluid. Test results between the two models (in vivo and in vitro) correlated well, with correlation coefficients of 0.85 for in vivo efficacy versus in vitro efficacy against suspended bacteria and 0.72 for in vivo efficacy versus in vitro efficacy against adherent bacteria ($P < 0.05$) for *Staphylococcus aureus*, 0.96 and 0.82 ($P < 0.05$) for *Staphylococcus epidermidis*, and 0.89 and 0.97 for *Escherichia coli*, respectively. In contrast, standard susceptibility tests, ratios of MICs to trough or peak levels, ratios of the area under the curve to the MIC, or time above the MIC were not predictive for therapeutic outcome in either the in vitro or in vivo model. In both models, the bactericidal activity levels with combination regimens were significantly higher than those with single-drug regimens ($P < 0.001$). Furthermore, rifampin combinations with either vancomycin, teicoplanin, fleroxacin, or ciprofloxacin were significantly more bactericidal against adherent bacteria than netilmicin combinations with vancomycin or daptomycin ($P < 0.01$). Thus, in vivo verification of the pharmacokinetic in vitro model correlated well with the animal model. The in vitro model offers an alternative to the animal model in experiments that screen and assess antibiotic regimens against device-related infections.

Device-related infections represent a major clinical issue. The present study focuses on the evaluation of a pharmacokinetic in vitro model in comparison to an animal model of such infections. Antibiotic therapy often fails without removal of the infected implant despite the use of drugs which are highly active in standard in vitro susceptibility tests (11). Poor therapeutic outcome of antibiotic treatment is related to compromised local host defense and to reduced antibiotic activity against adherent as opposed to suspended pathogens. Various animal models of implant-associated infections have been used to search for more-effective antimicrobial treatment of device-related infections (7, 31, 32). The tissue-cage guinea pig model stimulates human device-related infections in the sense that no spontaneous cure occurs (31, 32). This may be due to the previously described immune deficiency around the implant (30). A predictive in vitro model would allow the testing of a broad variety of new antimicrobial agents mimicking exposure to drugs according to human instead of animal kinetics and including antimicrobial agents that cannot be tested in some animal models, e.g., antibiotics with activity against anaerobic bacteria in guinea pigs.

In contrast to the antibiotic therapy of many bacterial infections, standard susceptibility in vitro tests are not predictive for the therapeutic outcome of device-related infections (26, 27). Recently, a pharmacodynamic model that quantitatively considers the activity of antimicrobial agents against both adherent and suspended bacteria, mimicking prolonged bacterial exposure to oscillating drug levels according to human serum kinetics, has been presented. Differences in response between suspended and adherent inocula have been found for some regimens and strains (22).

The usefulness of in vitro methods needs to be documented with verification studies, by comparing the in vitro data with results obtained for animals or in clinical studies. It was the purpose of this study to verify the recently developed pharmacodynamic in vitro model with an established in vivo model of device-related infection. The in vivo model selected has been previously described for both studying the pathogenesis of foreign body infection and assessing the efficacy of antibiotic therapies (26, 31). This verification study presents a quantitative comparison of drug efficacy in vivo and in vitro against both suspended and adherent bacteria. Using the same antibiotic regimens against the identical strains in both models, the study focused on staphylococci, the major cause of device-related infections (2, 13). In addition, the verification was also expanded with a limited number of experiments with a gram-negative pathogen.

MATERIALS AND METHODS

Bacteria. Two reference strains and one clinical isolate were used in the in vitro and in vivo experiments: *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, and *Staphylococcus epidermidis* B3972, a weak slime-producing clinical isolate from a blood culture of a patient with catheter-related septicemia (6, 26). Working cultures were maintained on blood agar and were transferred twice a month. The strains were stored at −70°C in skim milk and subcultured every 3 months.

Drugs. The following antimicrobial agents, kindly provided by the indicated manufacturers, were evaluated: ciprofloxacin (Bayer AG, Wuppertal, Germany), aztreonam (Squibb AG, Baar, Switzerland), co-trimoxazole (trimethoprim-sulfamethoxazole) and fleroxacin (Hoffmann-La Roche & Co., Basel, Switzerland), rifampin (Ciba-Geigy Ltd., Basel, Switzerland), netilmicin (Schering, Kenilworth, N.J.), vancomycin and daptomycin (Eli Lilly, Indianapolis, Ind.), teico-

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TABLE 1. Inoculum sizes in in vitro and animal models at start of antimicrobial therapy

Inoculum	Mean log CFU ± SD[a]		
	In vitro model		Tissue-cage model[b]
	Suspended bacteria	Adherent bacteria	
S. aureus ATCC 29213	7.4 ± 0.3	7.0 ± 0.3	6.2 ± 0.8
S. epidermidis B3972	7.4 ± 0.3	7.1 ± 0.3	5.0 ± 0.8
E. coli ATCC 25922	7.5 ± 0.4	6.8 ± 0.7	6.6 ± 1.3

[a] Log CFU/ml (suspended bacteria) or log CFU per bead (adherent bacteria).
[b] Log CFU/ml of tissue cage fluid; these colony counts correspond to the plateau of the bacterial density in the tissue cage abscesses.

TABLE 2. Peak and trough concentrations of drugs tested

Drug	In vitro model drug concn (mg/liter) in broth[a]:		Tissue-cage model drug concn (mg/liter) in[b]:		
			Serum	Cage fluid	
	Peak	Trough	Peak	Peak	Trough
Netilmicin	24	<0.1	67	36	7.3
Amikacin	25	2.2	46	7.4	4.7
Aztreonam	90	0.7	183	8.8	6.1
Ciprofloxacin	2	0.3	3.1	0.95	0.11
Fleroxacin	4.2	2.0	4.9	3.1	0.23
Vancomycin	25	7	22	8.6	2.8
Teicoplanin	14	7.8	15	9.6	7.7
Daptomycin	12.5	3.5	15	6.3	3.8
Rifampin	12	1.8	14.5	8.3	1.9
Co-trimoxazole[c]					
Trimethoprim	2	0.9	2.7	1.5	<0.5
Sulfamethoxazole	60	28	60	27	7.8

[a] Drugs were administered twice daily, except for netilmicin (once daily) and amikacin (three times a day).
[b] Drugs were administered twice daily (26, 27); netilmicin and amikacin were administered intramuscularly, and all other drugs were administered intraperitoneally.
[c] Tested in vitro at a ratio of 1:20.

planin (Merrell Dow Pharmaceuticals, Thalwil, Switzerland), and amikacin (Bristol Meyers, Baar, Switzerland). Doses and dosage intervals were chosen to achieve drug levels in the serum of the animal and in broth in vitro which mimic human serum kinetics during clinical treatment.

In vitro model. (i) System. A previously described pharmacokinetic in vitro model was used to expose bacterial cultures to oscillating concentrations of antimicrobial agents (22). In brief, 17-ml culture compartments were continuously perfused with tryptic soy broth (TSB) supplemented with 50 μg of Ca^{2+} per ml and 25 μg of Mg^{2+} per ml (TSB-S). In experiments with trimethoprim and sulfamethoxazole, TSB-S was replaced by Mueller-Hinton broth supplemented with 0.25% glucose. In addition, 250 U of thymidine phosphorylase (Sigma Chemie, Buchs, Switzerland) per ml was added since thymidine can reduce the activities of trimethoprim and sulfamethoxazole.

(ii) Suspended bacterium inocula. Volumes of 0.6 to 1 ml of overnight cultures were added into 17 ml of broth within the culture compartments. Specimens (0.3 ml) of the culture medium were sampled from each culture compartment seven times within the 48-h treatment period (at time zero and at 2, 6, 24, 26, 30, and 48 h) to document the number of CFU of suspended bacteria over time.

(iii) Adherent bacterium inocula. Inocula of adherent bacteria inocula were set as biofilm on 12 sinter glass beads (22). These beads consist of pieces of glass which have been pressed together to form a porous ball. Bacterial biofilms were obtained after incubation of 20 h in a bacterial culture. Sinter glass beads with adherent bacteria were exposed to oscillating antibiotic concentrations. One bead was removed from the culture compartment with a sterile surgical forceps at time zero and at 2, 6, 24, 26, 30, and 48 h and placed in tubes containing 2 ml of sterile broth. At least 90% of the adherent bacteria were detached from the glass surface by vigorously vortexing the tubes (22). The number of CFU of these suspended bacteria was determined.

Table 1 shows the inoculum sizes of both suspended and adherent bacteria. The response to the regimens was expressed as the reduction in the number of log CFU after 48 h of treatment. Efficacy was defined as the reduction in the log number of log CFU at the end of the 48-h treatment period compared with the number of log CFU at start of the treatment.

(iv) Dosage regimens in vitro. Antibiotic doses administered into the culture compartments were eliminated exponentially, because of continuous perfusion of the compartment. Concentration-time profiles were defined according to human serum kinetics by adjusting the appropriate flow rate of the pump. Drugs were administered as 1-h infusions, except for combination regimens in which daptomycin, vancomycin, and teicoplanin were administered as continuous infusions over 48 h. Concentrations of vancomycin, netilmicin, and amikacin achieved in the culture compartment were measured by fluorescence polarization immunoassay (TDx; Abbott Laboratories, Abbott Park, Ill.). The coefficient of variation of the fluorescence polarization immunoassay was <3%. The differences between the measured and the calculated concentrations of amikacin achieved in the culture compartment averaged 6% (mean of 24 measurements). These deviations account for the variations of the TDx assay as well as for mechanical instabilities of the in vitro system (in particular, the pumps). Table 2 summarizes peak and trough drug concentrations obtained in the pharmacodynamic model during the 48-h treatment period. Dosing intervals were selected to mimic peak and trough concentrations in serum achieved during clinical treatment.

Animal model. A previously described device-related animal model was used (30, 32). In brief, four sterile polytetrafluoroethylene (Teflon) tubes (32 by 10 mm) perforated with 130 regularly spaced 1-mm diameter holes (Ciba-Geigy Ltd.) were aseptically implanted in the flanks of albino guinea pigs (weight, 600 to 1,100 g). Experiments were started after complete healing of the wound, i.e., 3 to 6 weeks after surgery. Prior to each experiment, the interstitial fluid that accumulated in the tissue cages was checked for sterility. At time zero, tissue cages of the treatment group were infected by local inoculation of 10^4 CFU. Twenty-four hours after inoculation, antibiotic therapy was started. The inoculum sizes of suspended bacteria obtained at that time within the cages (Table 1) remained at a steady state for more than 1 week (27). The following drugs were given every 12 h for 4 days (total of eight doses): vancomycin (15 mg/kg of body weight), daptomycin (5 mg/kg), teicoplanin (6.7 mg/kg), netilmicin (20 mg/kg), ciprofloxacin (10 mg/kg), rifampin (25 mg/kg), fleroxacin (15 mg/kg against staphylococci and 10 mg/kg against *E. coli*), amikacin (20 mg/kg), aztreonam (200 mg/kg), and cotrimoxazole (10 or 50 mg/kg). Application was done intraperitoneally except for netilmicin and amikacin, which were given intramuscularly. Quantitative cultures of the tissue cage fluid were obtained on day 1 immediately before the onset of treatment and 2 days after the end of the 4-day treatment. The 2-day delay was chosen to avoid carryover of the antibiotic. The efficacy of each regimen was defined as the reduction in the log CFU count 2 days after treatment compared with the log CFU count at the start of treatment. Preliminary experiments revealed that the timing of the CFU count in the animals was not critical. Measurements obtained towards the end of the 4-day treatment were compared with data obtained 2 days after the end of antimicrobial therapy. The average difference between these two measurements was 0.51 log CFU (24 experiments with 296 samples of tissue cage fluid).

Drug levels were determined in sterile tissue cages. Peak drug levels in serum were determined 1 h after drug application. Peak levels in tissue cage fluid were measured 3 to 5 h after dosing. Preliminary experiments indicated that the concentrations obtained within this period are representative of the true peak (data not shown). Trough levels in cage fluid were measured at 12 h. The levels of all antibiotics, except co-trimoxazole, were determined by agar diffusion bioassays (10). All coefficients of variation were below 10.2%, as determined with 46 quality control specimens within the range of concentrations measured for the respective drugs. The following organisms were used as test strains for the indicated antibiotics: *Sarcina lutea* for rifampin; *Bacillus subtilis* (strain 0453-360; Difco, Detroit, Mich.) for ciprofloxacin, fleroxacin, vancomycin, teicoplanin, and netilmicin; *Micrococcus luteus* ATCC 9341 for daptomycin; and *E. coli* ATCC 25922 for aztreonam. Cotrimoxazole was determined by high-pressure liquid chromatography assay, kindly performed by Hoffmann-La Roche.

MICs. MICs were determined by the broth dilution method supplemented with appropriate divalent cations (18). The inoculum was adjusted from an overnight culture to a concentration of 5×10^5 CFU/ml.

Statistics. Geometric means were used to describe the average number of CFU determined in multiple experiments. If no CFU were detected in a 100-μl sample, a value of 10 CFU/ml was arbitrarily used for the calculation of the geometric mean. Efficacy was expressed as the mean reduction in log CFU counts at the end of treatment as a percentage of the log inoculum size. Final response was analyzed by logarithmic transformation of the CFU data followed by the Kruskal-Wallis test. Further comparisons, when appropriate, were done by the Bonferroni-adjusted Mann-Whitney test (19). Differences in mean reductions of log CFU counts between the two strains of staphylococci were analyzed by the Wilcoxon test. Correlation of efficacy in vivo versus in vitro against suspended and adherent bacteria was determined by Spearman's rank correlation analysis. The chi-square test was used for analysis of frequency of bacterial eradication. To calculate the time above the MIC or the area under the curve (AUC) divided by the MIC, a monoexponential decline of the concentration between the peak and trough levels was assumed. The increase in the concen-

TABLE 3. MICs and linked pharmacodynamic parameters of antimicrobial agents tested in this study

Inoculum	Antimicrobial agent	MIC (mg/liter)	Peak/MIC ratio		AUC/MIC ratio		Time above MIC (%)[a]	
			In vitro	In vivo	In vitro	In vivo	In vitro	In vivo
S. aureus ATCC 29213								
	Netilmicin	<0.25	>96	>144	>332	>922	66	100
	Ciprofloxacin	0.25	8	4	44	21	100	70
	Fleroxacin	0.5	8.4	6	71	31	100	77
	Vancomycin	1.0	25	9	172	64	100	100
	Teicoplanin	0.25	56	38	510	414	100	100
	Daptomycin	2.0	6.3	3	43	30	100	100
	Rifampin	0.016	750	519	4,128	3,445	100	100
S. epidermidis B3972								
	Ciprofloxacin	0.06	33	16	184	87	100	100
	Vancomycin	1.0	25	9	172	64	100	100
	Teicoplanin	2.0	7	5	64	44	100	100
	Daptomycin	1.0	13	6	86	60	100	100
	Rifampin	0.031	387	268	2,130	1,778	100	100
E. coli ATCC 25922								
	Amikacin	2	13	4	40	36	100	100
	Aztreonam	0.125	720	70	1,981	710	100	100
	Ciprofloxacin	0.02	100	48	550	262	100	100
	Fleroxacin	0.07	60	44	510	221	100	100
	Trimethoprim[b]	0.06	33	25	277	151	100	100
	Sulfamethoxazole[b]	1.2	50	23	422	161	100	100

[a] Time period for which the concentration was higher than the MIC.
[b] Co-trimoxazole, tested in vitro at a ratio of trimethoprim to sulfamethoxazole of 1:20.

tration from the trough level measured before drug administration to the peak determined after dosing was assumed to be linear over time.

RESULTS

Table 2 compares the peak and trough drug levels obtained in the in vitro model with the peak levels in serum and in cage fluid and trough levels in vivo. Table 3 summarizes the MICs and three pharmacodynamic parameters for the three pathogens. Ratios of peak levels to MICs ranged from 6 to 750 for the pharmacodynamic in vitro model and from 3 to 519 for the tissue-cage model. A similarly wide range was calculated for the ratios of AUC to MIC. In fact, both ratios were highly correlated ($P < 0.001$) for the regimens studied in vitro ($r = 0.96$) and in vivo ($r = 0.98$). Therefore, the study does not allow for a meaningful comparison of the predictive value of these two parameters relative to each other. Nevertheless, both parameters did not correlate significantly with the activity in the in vitro or in vivo model (Fig. 1 to 3). Poor predictive power was also observed for another pharmacodynamic parameter, the time above the MIC. Trough levels of all regimens in the animal model and in the in vitro model exceeded the MIC during all experiments with *S. epidermidis* and *E. coli* and also during most experiments with *S. aureus*, except for fleroxacin and ciprofloxacin (trough levels, 50 and 40% of the MICs, respectively) in the animal model and netilmicin (trough level, 40% of the MICs) in the in vitro model (Tables 2 and 3). Therefore, the time above the MIC was 100% during 33 of 36 single drug treatments in vitro or in vivo.

Similar responses to all drug regimens were found between *S. aureus* and *S. epidermidis* in the in vitro and also the in vivo models (Fig. 1 and 2). Combination regimens showed significantly higher levels of bactericidal activity in both models of infection than did single-drug regimens ($P < 0.001$). All rifampin combinations were significantly more active in vivo and

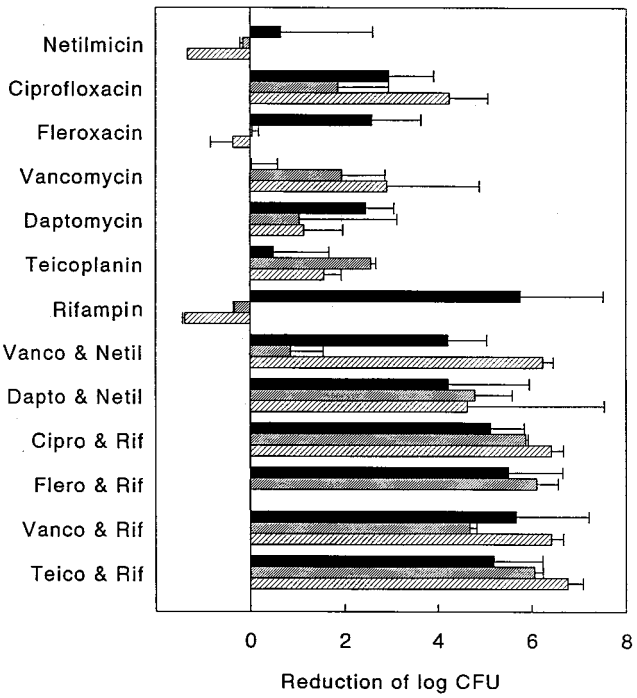

FIG. 1. Reductions in number of log CFU of suspended (▨) and adherent (▨) bacteria in vitro model and of bacteria in tissue-cage fluid in in vivo model (■) during treatment of *S. aureus* ATCC 29213 with 13 drug regimens. Geometric means for two or three replicates in the in vitro model and for 4 to 16 replicates in the in in vivo model are shown. Errors bars indicate standard deviations. Vanco, vancomycin; Netil, netilmicin; Dapto, daptomycin; Cipro, ciprofloxacin; Rif, rifampin; Flero, fleroxacin; Teico, teicoplanin.

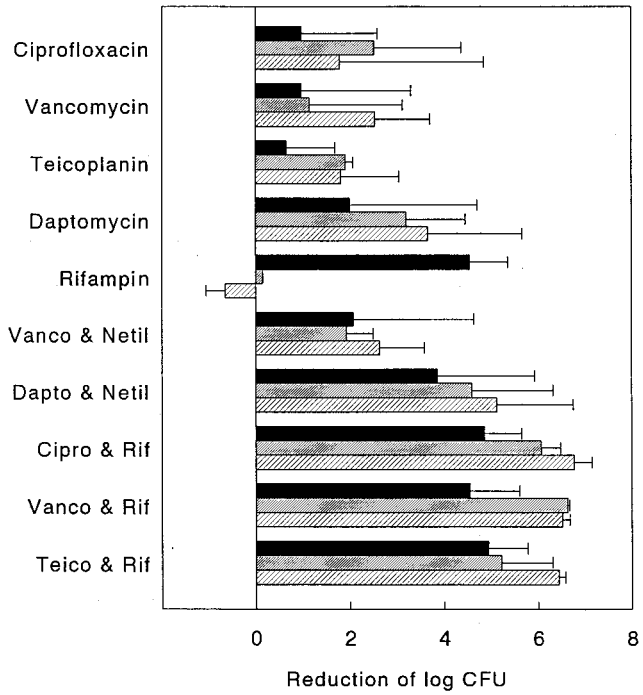

FIG. 2. Reductions in number of log CFU of suspended and adherent bacteria in in vitro model and of bacteria in tissue-cage fluid in in vivo model during treatment of *S. epidermidis* B3972 with 10 drug regimens. Geometric means for 2 to 4 replicates in the in vitro model and for 12 replicates in the in vivo model are shown. Error bars indicate standard deviations. Abbreviations and symbols are defined in the legend to Fig. 1.

in vitro against adherent bacteria than were netilmicin combinations ($P < 0.01$).

Correlation of in vivo efficacy versus in vitro efficacy against suspended or adherent bacteria of *E. coli* resulted in correlation coefficient values of 0.89 and 0.97, respectively. The respective coefficients for staphylococci ranged from 0.35 to 0.59. The low coefficient values obtained with both staphylococci related to a major discrepancy occurring during single drug treatment with rifampin. Resistance developed rapidly in vitro but not in vivo, probably because of the higher inoculum concentration used in vitro compared with that used in vivo (Table 1). In additional experiments using lower concentrations of suspended and adherent bacteria in the inocula (5.9 CFU/ml and 3.9 log CFU per bead for *S. aureus* and 5.6 log CFU/ml and 5.6 log CFU per bead for *S. epidermidis* B3972, respectively), rifampin eradicated inocula of both strains and by both modes of inoculation in vitro, improving the correlation coefficients to 0.85 and 0.72 for *S. aureus* and 0.96 and 0.82 for *S. epidermidis*, respectively ($P < 0.05$). Exclusion of the rifampin single-drug treatments resulted in similar correlation coefficients of 0.84 and 0.66 for *S. aureus* and 0.97 and 0.80 for *S. epidermidis* ($P < 0.05$). The other major discrepancy between in vivo and in vitro efficacy occurred with the vancomycin-netilmicin combination against adherent *S. aureus* (Fig. 1). This discrepancy might relate to higher levels of netilmicin in vivo than in vitro. In additional in vitro experiments with the vancomycin-netilmicin combination, simulating in vitro drug levels equal to those measured in vivo in tissue cages, a reduction of 5.5 log CFU was obtained in vitro. This was similar to the 4.2 log reduction in vivo and improved the correlation coefficient to 0.83.

DISCUSSION

This paper describes in vitro studies in a recently developed pharmacodynamic model which allows the exposure of suspended and adherent bacterium inocula to drug regimens simulating human serum kinetics (22). Identical regimens against the same strains were tested in both the in vitro and in vivo models of device-related infections. In contrast to the in vitro model, leukocytes, antibodies, complements, and other host defenses played a role in determining the magnitude of reduction of CFU counts in vivo. Basically similar reductions in log CFU counts have been observed in both models with all except one regimen against the tested strains. The major discrepancy was observed for rifampin, which showed high levels of bactericidal activity in the animal model but was inactive because of the emergence of resistance in the in vitro model. This may be due to host defense mechanisms or, alternatively, to lower inoculum concentrations in vivo. Indeed, eradication of staphylococci by rifampin has been confirmed in vitro by using reduced inoculum concentrations. Nevertheless, the favorable results obtained during single-drug therapy with rifampin in vivo may be misleading for clinical use, which represents the gold standard for any model of drug activity. Rapid development of resistance may occur by single step mutation, depending on the inoculum size (24). In vitro (14), animal model (16, 17, 29), and clinical failures (4, 5, 8, 20, 21) have been reported for treatment with rifampin. Therefore, rifampin combinations with vancomycin, teicoplanin, ciprofloxacin, and fleroxacin have been evaluated to prevent emergence of rifampin resistance. All combinations with rifampin showed effective bactericidal activity, resulting in efficacies of 79 to 100% in vivo and 81 to 100% in vitro. They were significantly more active against adherent staphylococci of both strains compared with netilmicin-vancomycin and netilmicin-daptomycin combinations ($P < 0.01$).

Correlation coefficients of in vivo versus in vitro efficacy against either suspended or adherent bacteria were similar.

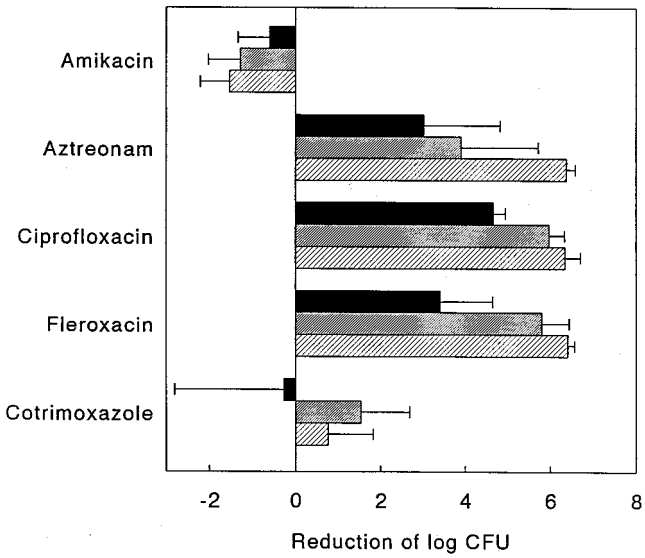

FIG. 3. Reductions in number of log CFU of suspended and adherent bacteria in in vitro model and of bacteria in tissue-cage fluid in in vivo model during treatment of *E. coli* ATCC 25922 with five drug regimens. Geometric means for 3 replicates in the in vitro model and for 12 replicates in the in vivo model are shown. Error bars indicate standard deviations. Symbols are defined in the legend to Fig. 1.

Nevertheless, a tendency towards higher levels of antibacterial activity against suspended than against adherent inocula was noticed. The frequency of experiments with eradication was higher in the presence of suspended bacterium inocula. Twenty-eight of 67 treatments (42%) eradicated suspended bacteria below the detection limit compared with 20 of 74 treatments (27%) against adherent bacteria ($P = 0.06$). However, discrepancies of >2 log CFU in bacterial killing of suspended or adherent pathogens were limited to *E. coli* exposed to aztreonam and to *S. aureus* exposed to ciprofloxacin or to vancomycin plus netilmicin.

Ratios of drug levels to MICs (3, 9, 15) or in vitro susceptibility (12, 23) have been shown to be predictive for the outcome of antibiotic therapy of various bacterial infections. For the treatment of device-related infections, however, correlation between the in vivo efficacy of antibiotics and the ratios of drug levels to MICs is poor (26, 27). In the present study, netilmicin administered alone could not reduce bacterial inocula of *S. aureus* in the in vitro model despite peak-to-MIC ratios of above 96, and only a 0.65 log CFU reduction occurred in vivo during the 4 days of treatment despite peak-to-MIC ratios of >144. Drug levels higher than the MIC were also obtained with amikacin against *E. coli*. Nevertheless, amikacin was completely ineffective against *E. coli*. Teicoplanin, a glycopeptide with a high level of activity against staphylococci (1), produced only limited killing in both models, despite the fact that even the trough levels in both models exceeded the MIC 31 times. Similar results were obtained with vancomycin, the other glycopeptide studied. The lack of activity of co-trimoxazole against *E. coli* despite high peak-to-MIC levels in the animal model has been confirmed by the in vitro model. This confirms its clinical failure in device-related salmonella infection (25).

Single-drug regimens against staphylococci were significantly less active than combination regimens ($P < 0.001$) in both models. None of the tested single-drug regimens eradicated inocula of staphylococci either in the pharmacodynamic in vitro model or in the animal tissue-cage model, except rifampin in vivo. Effective bactericidal activities of the quinolones ciprofloxacin and fleroxacin against *E. coli* have been observed in both the in vitro and in vivo models. In contrast, aztreonam could not eradicate adherent *E. coli* in either model, and neither amikacin nor co-trimoxazole was bactericidal.

The use of animal models in research and development has been criticized for ethical reasons. Therefore, the scientists in industry and academia have been challenged to intensify the search for alternatives to reduce, refine, and replace animal experiments (28). This validation study demonstrated good correlation of bacterial killing between the in vitro and the in vivo models. Nevertheless, some discrepancies in in vivo and in vitro efficacies were noted. There is only limited clinical information available for deciding which model more closely reflects the situation in humans, e.g., for the most-pronounced discrepancies found, the activity of single-drug treatments with rifampin. In conclusion, the pharmacokinetic in vitro model offers an alternative to animal models of device-related infections for antibiotic testing, including the screening of new compounds.

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