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Addition of Gentamicin or Rifampin Does Not Enhance the Effectiveness of Daptomycin in Treatment of Experimental Endocarditis Due to Methicillin-Resistant *Staphylococcus aureus*▽

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This study evaluated the activity of daptomycin combined with either gentamicin or rifampin against three methicillin-resistant *Staphylococcus aureus* (MRSA) clinical isolates in vitro and one isolate in vivo against a representative strain (MRSA-572). Time-kill experiments showed that daptomycin was bactericidal against these strains at concentrations over the MIC. Daptomycin at sub-MIC concentrations plus gentamicin at 1× and 2× the MIC yielded synergy, while the addition of rifampin at 2 to 4 μg/ml resulted in indifference (two strains) or antagonism (one strain). The in vivo activity of daptomycin (6 mg/kg of body weight once a day) was evaluated ± gentamicin (1 mg/kg intravenously [i.v.] every 8 h [q8h]) or rifampin (300 mg i.v. q8h) in a rabbit model of infective endocarditis by simulating human pharmacokinetics. Daptomycin plus gentamicin (median, 0 [interquartile range, 0 to 2] $\log_{10}$ CFU/g vegetation) was as effective as daptomycin alone (0 [0 to 2] $\log_{10}$ CFU/g vegetation) in reducing the density of bacteria in valve vegetations ($P = 0.83$), and both were more effective than daptomycin plus rifampin (3 [2 to 3.5] $\log_{10}$ CFU/g vegetation; $P < 0.05$) for the strain studied. In addition, daptomycin sterilized a ratio of vegetations that was similar to that of daptomycin plus gentamicin (10/15 [67%] versus 9/15 [60%]; $P = 0.7$), and both regimens did so more than daptomycin plus rifampin (3/15 [20%]; $P = 0.01$ and $P = 0.02$, respectively). No statistical difference was noted between daptomycin plus gentamicin and daptomycin alone for MRSA treatment. In the combination arm, all isolates from vegetations remained susceptible to daptomycin, gentamicin, and rifampin. Sixty-one percent of the isolates (8/13) acquired resistance to rifampin during monotherapy. In the daptomycin arm, resistance was detected in only one case, in which the daptomycin MIC rose to 2 μg/ml among the recovered bacteria. In conclusion, the addition of gentamicin or rifampin does not enhance the effectiveness of daptomycin in the treatment of experimental endocarditis due to MRSA.

Staphylococcus aureus is a common cause of infective endocarditis (IE), with methicillin-resistant *S. aureus* (MRSA) strains found in up to one-third of all cases (11, 28). Due to multidrug resistance among many strains, vancomycin is the standard therapy for IE caused by MRSA (1). However, vancomycin therapy has been associated with poor outcomes that may be explained by the drug's slow bactericidal activity and insufficient diffusion into valve vegetations (5, 10, 23).

Daptomycin is a cyclic lipopeptide that is rapidly bactericidal against gram-positive pathogens such as MRSA, including strains that exhibit resistance to vancomycin. It is approved for the treatment of skin and soft tissue infections, *S. aureus* bacteremia, and right-sided native valve endocarditis (6). However, there is limited information regarding the efficacy of daptomycin in the treatment of left-sided native valve IE caused by MRSA. In a randomized clinical trial (10), none of the patients with left-sided endocarditis treated with daptomycin at 6 mg/kg of body weight/day were cured, and postmarketing registry data (24) revealed a successful clinical outcome in only 9 out of 15 cases (60%).

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Therefore, given the lack of efficacy data with daptomycin monotherapy in left-sided MRSA endocarditis, the continued evaluation of methods to enhance the activity of daptomycin is warranted. It is unknown whether daptomycin's activity against MRSA may be improved by combining it with one or more additional antibiotics to produce a potentially additive or synergistic effect. Gentamicin has been shown to augment daptomycin's activity against strains of MRSA in vitro (4, 20, 35). The combination of daptomycin plus rifampin has demonstrated additive activity against MRSA in vitro (4) and has enhanced activity against MRSA in vivo (4, 32). The aim of this study was to evaluate the in vitro activity of daptomycin combined with gentamicin or rifampin against MRSA and compare treatment with daptomycin alone to treatment with both combinations in experimental MRSA aortic valve endocarditis using a human-adapted pharmacokinetic model.

(This work was previously presented at the 47th Interscience Conference on Antimicrobial Agents and Chemotherapy [ICAAC], Chicago, IL, 17 to 20 September 2007 [29a] and at the 48th Annual ICAAC-IDSA Annual Meeting, Washington, DC, 25 to 28 October 2008 [29b].)

MATERIALS AND METHODS

Bacterial isolates. The three MRSA strains (MRSA-564, MRSA-572, and MRSA-593) used in the study described here were isolated from patients with endocarditis at Hospital Clínic Universitari in Barcelona, Spain. One of them was used for the in vivo study (MRSA-572). They were stored at −80°C in skim milk.

Antibiotics. Daptomycin powder was supplied by Cubist Pharmaceuticals (Lexington, MA). Gentamicin and rifampin were purchased from Sigma (St. Louis, MO).

Susceptibility testing. Daptomycin, gentamicin, and rifampin MICs and minimum bactericidal concentrations (MBCs) were determined using the microdilution method in liquid medium cation-adjusted Mueller-Hinton broth (Oxoid, Ltd., Hampshire, England), according to the procedures of the Clinical and Laboratory Standards Institute (CLSI) (30). Daptomycin susceptibility testing was performed in Mueller-Hinton broth adjusted to 50 μg/ml of calcium per standard methodology. *S. aureus* ATCC 29213 was used as the test control strain.

Time-kill studies. Time-kill methodology was used to test the activity of daptomycin plus gentamicin or rifampin against MRSA strains according to previously described criteria (9). A final inoculum between 5×10^5 and 1×10^6 CFU/ml was used. Prior to inoculation, each tube of fresh cation-adjusted Mueller-Hinton broth was supplemented with daptomycin alone or in combination with gentamicin or rifampin. Concentrations between 0.5× and 2× the MIC for daptomycin, between 2 and 4 μg/ml for rifampin, and between 0.5 and 2.0 μg/ml for gentamicin were chosen for synergy testing. A tube without antibiotic was used as a growth control. Viability counts were performed at 0, 4, and 24 h, as per the recommendation of H. Isenberg et al. (18). Drug carryover was addressed by dilution. Synergy was defined as a $\geq$2-$\log_{10}$ decrease in the number of CFU/ml between the test tube with the combination and the test tube with the most active agent alone after 24 h: the number of surviving organisms in the presence of the combination had to be $\geq$2 $\log_{10}$ CFU/ml below the starting inoculum. At least one of the drugs had to be present in a concentration that did not significantly affect the growth curve of the test organism when used alone (9). Antagonism was defined as a $\geq$2-log increase in the number of CFU/ml between the test tube with the combination and the test tube with the most active agent alone after 24 h. Bactericidal activity was defined as at least a 3-log reduction in CFU at 24 h in comparison with the initial inoculum.

Study animals. An experimental aortic valve endocarditis was induced in New Zealand White rabbits (body weight, 2 kg), which were obtained from San Bernardo Farm (Pamplona, Spain). The animals were housed in the animal facilities of the University of Barcelona, School of Medicine, which is equipped with automatic air exchange with a HEPA filter and a circadian light cycle. They were nourished ad libitum. This study was approved by the Ethical Committee of Experimental Animal Studies of the University of Barcelona.

Human pharmacokinetics simulation studies. Antibiotics were administered using a computer-controlled infusion pump system, designed to reproduce human serum pharmacokinetics in rabbits after an intravenous (i.v.) infusion. Animal drug doses were chosen to simulate the pharmacokinetic profile of daptomycin (6 mg/kg i.v. once a day [q.d.]) (6, 7), gentamicin (1 mg/kg i.v. every 8 h [q8h]), and rifampin (300 mg i.v. q8h) in humans. Gentamicin and rifampin doses were chosen based upon endocarditis treatment guidelines from the American Heart Association and the European Society of Cardiology (1, 17). The daptomycin dose was derived from the randomized clinical trial of daptomycin versus standard therapy for *S. aureus* bacteremia and endocarditis (1, 10). To determine the concentrations of the antibiotics in serum samples in five healthy rabbits, blood was drawn from a carotid catheter at different times after a single i.v. injection of the antibiotic. Rabbits received 15 mg/kg of daptomycin, 1 mg/kg of gentamicin, and 15 mg/kg of rifampin. Daptomycin concentrations in plasma were assayed by the disk-plate bioassay method (25). Rifampin and gentamicin concentrations were assayed at the Centre de Diagnòstic Biomèdic, Hospital Clínic, Barcelona. Gentamicin concentrations were measured by the Aca Genta method, an immunoassay with Emit technology (Behring Diagnostics, San Jose, CA). The limit of detection of the procedure was 0.5 μg/ml. A rapid, simple, and sensitive high-performance liquid chromatography assay was developed for the quantification of rifampin in rabbit plasma. The method involved the solid-phase extraction with C_{18} cartridges of the drug from 400 μl of rabbit plasma. Ascorbic acid was added during drug extraction to prevent degradation of rifampin. The high-performance liquid chromatography analysis used a symmetry C_{18} analytical column and a mobile phase consisting of a mixture of acidified water with phosphoric acid (pH 2.4) and acetonitrile (55/45 [vol/vol]) and with UV monitoring at 333 nm. The method was successfully validated. The method was linear over the concentration range studied (0.1 to 20 μg/ml). Analytical results of calibration standards in terms of percent precision and percent accuracy fulfilled all of the acceptance criteria. The overall precision and accuracy measurements, expressed as percent coefficient of variation and as percent mean deviation, were $\leq$15%, in terms of absolute values. The lower limit of quantification was 0.1 μg/ml for 400 μl of rabbit plasma (3, 21, 22).

In vivo experimental pharmacokinetic studies were performed in healthy rabbits to simulate human antibiotic pharmacokinetic profiles with an open one- or two-compartment model (15, 29). The pump system was set up to deliver an i.v. infusion into rabbits at previously calculated flow rates to simulate the human kinetics of 6 mg/kg daptomycin daily ($n = 5$), 1 mg/kg gentamicin q8h ($n = 5$), or 300 mg rifampin q8h ($n = 5$). To determine the antibiotic concentrations in rabbits' serum, 1 ml of blood was sampled at different times after the start of drug infusion.

Endocarditis model. Experimental aortic valve IE was induced according to the method described by Garrison and Freedman (12). A catheter was inserted through the right carotid artery into the left ventricle, and the catheter for the antibiotic administration was placed into the inferior vena cava through the jugular vein (29). The infusion pump delivered 2 ml/h of 0.9% saline solution until the beginning of antimicrobial administration. Twenty-four hours after placement of the intracardiac catheter, all animals were infected via the marginal ear vein with 1 ml of saline solution containing about 5×10^5 CFU/ml of the MRSA-572 strain. One milliliter of blood was obtained 18 h after infection and immediately before the initiation of antimicrobial therapy to confirm the bacteremia, which was interpreted to indicate IE. Antibiotic treatments were then initiated with combination therapy regimens (daptomycin plus gentamicin or daptomycin plus rifampin) or as monotherapy with either gentamicin, rifampin, or daptomycin. In combination regimens, daptomycin was administered first over an infusion period of 15 min, after which either rifampin or gentamicin was infused over 30 min. Also at that time, control animals were sacrificed and vegetations were quantified for bacterial CFU. Antibiotics were administered via the computer-controlled infusion pump system for 48 h. After finishing the 48-h treatment, animals were sacrificed after an additional 6 half-lives had elapsed. This provided growing time for residual viable bacteria contained within the endocardial vegetations.

Analysis of endocardial vegetations. After antibiotic treatment, rabbits were anesthetized and then sacrificed. Aortic valve vegetations were removed and processed (29). The results were expressed as the number of $\log_{10}$ CFU per gram of vegetation. A result was assigned a value of 2 $\log_{10}$ if there was no growth on the quantitative plates cultured with 100 μl from each dilution but there was growth in the qualitative culture (the rest of the homogenate in tryptic soy broth). The result was assigned a value of 0, and the vegetation was considered sterile if there was no growth from the initial quantitative culture and from the homogenates cultured for a week.

PAP studies. Isolates recovered from vegetations were frozen and stored at −80°C in skim milk. Before testing, each isolate was subcultured at least twice on blood agar plates to ensure optimal growth. The recovered bacteria were retested to measure daptomycin, gentamicin, and rifampin MICs for comparison to pre-

TABLE 1. MICs and MBCs of daptomycin, gentamicin, and rifampin for the three staphylococcal strains tested

Strain	Daptomycin		Gentamicin		Rifampin	
	MIC (mg/liter)	MBC (mg/liter)	MIC (mg/liter)	MBC (mg/liter)	MIC (mg/liter)	MBC (mg/liter)
MRSA-564	0.5	0.5	0.5	1	0.008	>0.25
MRSA-572	0.5	0.5	1	1	0.015	>0.25
MRSA-593	1	1	0.5	0.5	0.008	>0.25

treatment MICs. Moreover, daptomycin and vancomycin population analysis studies were done.

The modified population analysis profile (PAP) was determined as follows. Fresh cultures were diluted in saline from 10^2 to 10^8 CFU/ml and plated on brain-heart infusion agar plates (Oxoid, Ltd., Hampshire, England) containing 0, 0.5, 1, 2, 4, and 6 mg/liter vancomycin or plated on Mueller-Hinton agar plates (Oxoid, Ltd., Hampshire, England) containing 0, 0.25, 0.5, 1, 2, and 4 mg/liter daptomycin and supplemented with 50 μg/ml of calcium. Colonies were counted after 48 h of incubation at 37°C, and the viable count was plotted against the vancomycin or daptomycin concentration (37) and compared with that of the initial strain. ATCC 29213 was included as a control strain.

Statistical analysis. The results were expressed as the median and the interquartile range (IQR) interval of the number of $\log_{10}$ CFU/g vegetation. The Mann-Whitney rank sum test was used to compare the $\log_{10}$ CFU/g values between the different treatment groups. Fisher's exact test was used to compare the rate of sterilization of vegetations and to assess whether there were differences between treatment groups.

RESULTS

Susceptibility testing. The MICs and MBCs of the MRSA strains are shown in Table 1. All were susceptible to daptomycin, gentamicin, and rifampin, according to the CLSI standard MIC breakpoints (30).

In vitro time-kill experiments. The in vitro activity of daptomycin plus gentamicin or rifampin for the selected strains is presented in Tables 2 and 3. At the concentrations tested (between 0.5× and 2× the MIC for daptomycin and between 0.5× and 1× the MIC for gentamicin), daptomycin plus gentamicin (Table 2) showed synergy and, in most cases, demonstrated bactericidal activity for the combinations tested. Daptomycin plus rifampin (Table 3) showed indifference at concentrations tested for strains MRSA-564 and MRSA-572 but showed antagonism for MRSA-593.

Pharmacokinetic studies. To ensure that rabbits were dosed appropriately in the experimental IE model, it was necessary to first obtain the pharmacokinetic parameters of daptomycin, gentamicin, and rifampin in healthy, uninfected rabbits. Different pharmacokinetic parameters were estimated on the basis of an open two-compartment model to compare the pharmacokinetics in rabbits, the human-adapted model, and humans (13). Results are shown in Table 4. A computer-controlled infusion pump system produced serum kinetics in rabbits similar to those found in humans for daptomycin, gentamicin, and rifampin. The pharmacokinetic parameters obtained from the human-adapted model were similar to those in humans (2) (Table 4).

Treatment of established endocarditis. The relative effectiveness of the different daptomycin therapeutic regimens for the treatment of established endocarditis (daptomycin plus gentamicin and daptomycin plus rifampin) and the monotherapy regimens (daptomycin, gentamicin, or rifampin alone) are shown in Table 5. One rabbit treated with daptomycin plus gentamicin, two treated with daptomycin plus rifampin, and three treated with rifampin died during the experiment and were not included in the analyses.

TABLE 2. Time-killing curves of daptomycin plus gentamicin for the strains in the in vitro study

Strain and antibiotic(s) tested[a]	$\log_{10}$ CFU/ml at baseline	Change in $\log_{10}$ CFU/ml at:	
		4 h	24 h
MRSA-564			
Control	6	+2.1	+3
D 0.25	6	+0.6	+2.9
D 0.5	6	−1.3	+2.7
G 0.5	6	−0.4	+2.8
G 1	6	−0.4	+2.7
D 0.25 + G 0.5	6	−1.5	−1.2
D 0.25 + G 1	6	−1.5	−1.8
D 0.5 + G 0.5	6	−1.6	−4
D 0.5 + G 1	6	−1.6	−4
MRSA-572			
Control	6.1	+1.6	+2.8
D 0.5	6.1	−1	−0.1
D 1	6.1	−1.4	−1.2
G 1	6.1	−1.2	+2
G 2	6.1	−1.4	−0.3
D 0.5 + G 1	6.1	−2.2	−1.4
D 0.5 + G 2	6.1	−2.3	−4.1
D 1 + G 1	6.1	−2	−4
D 1 + G 2	6.1	−2.8	−4.1
MRSA-593			
Control	6.2	+1.6	+2.9
D 0.5	6.2	+0.3	+2.7
D 1	6.2	−1.4	−1.3
G 0.5	6.2	−2.5	+2.6
G 1	6.2	−2.6	+2.6
D 0.5 + G 0.5	6.2	−2.1	−4
D 0.5 + G 1	6.2	−2.1	−4
D 1 + G 0.5	6.2	−3	−4.2
D 1 + G 1	6.2	−3.4	−4.2

[a] D, daptomycin; G, gentamicin. The D and G numbers shown represent the concentration of each antibiotic in μg/ml.

All control rabbits infected with the test strain had infected aortic valve vegetations with a high median bacterial titer per gram of vegetation (≥9 $\log_{10}$ CFU). Comparisons between treated groups revealed that after 48 h of treatment, daptomycin plus gentamicin was as effective as daptomycin monotherapy in sterilizing the vegetations ($P = 0.7$). In comparison with daptomycin plus rifampin, daptomycin alone or daptomycin plus gentamicin sterilized significantly more vegetations ($P = 0.01$ and $P = 0.02$, respectively). Daptomycin monotherapy or combined with gentamicin reduced the median number of CFU in the vegetations of treated animals to a greater extent than when combined with rifampin. These differences were statistically significant ($P = 0.02$ and $P = 0.04$, respectively).

All isolates from the endocardial vegetations, except one from the daptomycin monotherapy arm, had the same daptomycin MIC as the initial infecting strain after daptomycin or daptomycin plus gentamicin or rifampin treatments. For the isolate from the monotherapy arm, the daptomycin MIC increased to 2 μg/ml, and the PAP studies showed subpopulations growing at concentrations of 4 μg/ml (9×10^4 CFU/ml). In the rifampin monotherapy arm, 61% (8/13) of the isolates developed resistance to rifampin. No resistance was detected in the gentamicin arm.

TABLE 3. Time-killing curves of daptomycin plus rifampin for the strains in the in vitro study

Strain and antibiotic(s) tested (μg/ml)[a]	Log_{10} CFU/ml at baseline	Change in log_{10} CFU/ml at:	
		4 h	24 h
MRSA-564			
Control	6	+2.1	+2.8
D 0.25	6	−1.3	+2.7
D 0.5	6	−1.8	−2.4
R 2	6	−0.4	−1.5
R 4	6	−0.5	−1.6
D 0.25 + R 2	6	−0.6	−2
D 0.25 + R 4	6	−0.6	−3
D 0.5 + R 2	6	−0.8	−2
D 0.5 + R 4	6	−0.8	−3.2
MRSA-572			
Control	5.9	+1.6	+2.9
D 0.5	5.9	−1.1	+1.5
D 1	5.9	−1.9	−2.7
R 2	5.9	−0.3	−1.3
R 4	5.9	−0.3	−1.2
D 0.5 + R 2	5.9	−0.2	−1
D 0.5 + R 4	5.9	−0.2	−0.9
D 1 + R 2	5.9	−0.7	−1.8
D 1 + R 4	5.9	−0.9	−1.9
MRSA-593			
Control	6.1	+1.9	+2.8
D 1	6.1	−2.1	−1.1
D 2	6.1	−3.9	−3.3
R 2	6.1	−0.1	−0.9
R 4	6.1	0	−1
D 1 + R 2	6.1	−0.2	−1
D 1 + R 4	6.1	−0.1	−1.1
D 2 + R 2	6.1	−0.2	−1
D 2 + R 4	6.1	−0.4	−1.1

[a] D, daptomycin; R, rifampin. The D and R numbers shown represent the concentrations of each antibiotic in μg/ml.

TABLE 5. Treatment of experimental endocarditis caused by MRSA-572

Treatment group	No. of animals with sterile vegetation/ total (%)[b]	Median (range) IQR (log_{10} CFU/g of vegetation)[b]
Control[a]	0/15 (0)	10 (9.7–10)
Gentamicin	0/12 (0)	8.6 (8.1–9)
Rifampin	0/13 (0)	6.6 (5.2–10)
Daptomycin	10/15 (67)*†	0 (0–2)‡§
Daptomycin + gentamicin	9/15 (60)*¶	0 (0–2)‡‖
Daptomycin + rifampin	3/15 (20)†¶	3 (2–3.5)§‖

[a] The control animals were sacrificed 18 h after the infection was started.
[b] *, $P = 0.70$; †, $P = 0.01$; ‡, $P = 0.83$; §, $P = 0.02$; ¶, $P = 0.02$; ‖, $P = 0.04$. The symbols represent levels of statistical significance between two values with the same symbol.

DISCUSSION

Daptomycin is an important new option for the treatment of severe infections caused by MRSA, including endocarditis. Although the addition of agents such as gentamicin or rifampin has long been used to enhance the effectiveness of standard therapy with vancomycin, the role of combination therapy with daptomycin in this area is still unclear.

The time-kill study results presented here indicate that sub-MIC concentrations of daptomycin plus gentamicin at concentrations at the MIC and 2× the MIC produce synergistic activity against the clinical strains of MRSA tested. However, the addition of rifampin at concentrations between 0.5 and 4 μg/ml produced indifference in two of the three strains tested. The third strain shows antagonism. The effect of daptomycin combined with gentamicin seen in our study is similar to the findings of Snydman et al. (34), who noted a synergistic effect in time-kill studies against MRSA with daptomycin plus genta-

TABLE 4. Pharmacokinetic parameters

Parameter[a]	Result for:		
	Daptomycin	Gentamicin	Rifampin
Previously reported human values (single dose)			
Dose	6 mg/kg i.v.[b]	1 mg/kg i.v.[c]	300 mg i.v.[d]
C_{max}/C_{min} (μg/ml)	86/15	10/1	6/0.9
k_{el} (h^{-1})	0.07[b]	0.25	0.23
$t_{1/2}$ β (h)	9.5	2.73	3
AUC (μg · h/ml)	973.9[b]	NA[e]	21.9
Protein binding	92%	NA	NA
Animal values ($n = 5$)			
k_{el} (h^{-1} [mean ± SD])	0.19 ± 0.01	0.78 ± 0.07	0.38 ± 0.04
$t_{1/2}$ β (h [mean ± SD])	3.57 ± 0.19	0.89 ± 0.08	1.84 ± 0.02
AUC (μg · h/ml [mean ± SD])	439.8 ± 132.4	6.36 ± 08	11.6 ± 2.1
Protein binding (%)	90	NA	NA
Human-like values in animals ($n = 5$)			
C_{max}/C_{min} (μg/ml [mean ± SD])	86/15	9.5 ± 2.1/1.4 ± 0.2	6.9 ± 0.47/1.3 ± 0.26
k_{el} (h^{-1} [mean ± SD])	0.09 ± 0.01	0.23 ± 0.02	0.24 ± 0.04
$t_{1/2}$ β (h [mean ± SD])	7.6 ± 1.3	3.04 ± 0.29	2.91 ± 0.47
AUC (μg · h/ml [mean ± SD])	845.7 ± 207	14.8 ± 0.6	18.32 ± 1.9

[a] C_{max}/C_{min}, maximum/minimum concentration of drug in serum; k_{el}, elimination rate constant; SD, standard deviation; $t_{1/2}$ β, terminal half-life; AUC, area under the concentration-time curve.
[b] Cubist Pharmaceuticals data on file (6) and data from Benvenuto et al. (2).
[c] Data from Gavaldá et al. (14).
[d] Data from Mensa et al. (27).
[e] NA, not available.

micin at 1/8 and 1/4 MIC. Credito et al. (4) also demonstrated synergy between daptomycin sub-MICs and gentamicin at concentrations below the MIC via time-kill methods among numerous community-acquired and nosocomial MRSA strains. Studies that have used time-kill assays to evaluate the combination of daptomycin and rifampin have shown either additive or antagonistic effects against both rifampin-susceptible and nonsusceptible strains of MRSA (4, 19).

LaPlante and Rybak (20) examined daptomycin in combination with low-dose gentamicin in the setting of high-inoculum MRSA in an in vitro pharmacodynamic model with simulated endocardial vegetations. In their study, gentamicin enhanced the bactericidal activity of daptomycin, as demonstrated by a more rapid time to 99.9% kill. A subsequent study that used the same model (35) found that the addition of gentamicin enhanced the activity of daptomycin and noted that this effect occurred with both low-dose (1 mg/kg q8h) and high-dose (5 mg/kg q24h) gentamicin regimens administered for either 24 or 96 h. Finally, Rose et al. (31) also used an in vitro pharmacodynamic model to evaluate daptomycin plus high-dose gentamicin and daptomycin plus rifampin against strains of MRSA with reduced daptomycin susceptibility. Their findings indicate variable activity, with increased killing with either combination in some but not all of the strains tested.

Although a number of studies have examined the effects of daptomycin plus gentamicin or rifampin in vitro, there are limited data about the in vivo efficacy of these combinations. The present study used a rabbit model and a dosing method designed to reproduce human pharmacokinetics to determine the effects of daptomycin (6 mg/kg q.d.) plus either gentamicin (1 mg/kg q8h) or rifampin (300 mg i.v. q8h) in the treatment of experimental aortic valve MRSA endocarditis. To our knowledge, this is the first study to examine the combination of daptomycin and gentamicin in an in vivo evaluation of experimental endocarditis. Our results indicate that there is no difference between therapy with daptomycin alone and that with daptomycin plus gentamicin, as both regimens produced similar reductions in the density of bacteria in vegetations ($P = 0.83$) and sterilized a similar proportion of vegetations ($P = 0.7$). Although these findings contrast the results of our time-kill assays, they are consistent with the only other in vivo study of this combination against MRSA, in which serum bactericidal titers from healthy human volunteers showed that the addition of gentamicin at 1 mg/kg q8h did not alter the area under the bactericidal curve of standard-dose daptomycin (6 mg/kg q.d.) against MRSA (8). However, given the in vitro studies indicating enhanced, more rapidly bactericidal activity of daptomycin plus gentamicin at various doses up to 5 mg/kg q.d. (20, 31, 35), further animal studies will be required to compare the effects of different gentamicin dosage regimens in combination with daptomycin.

The addition of rifampin did not improve daptomycin activity in our rabbit model. In agreement with the indifference or antagonism noted in our time-kill studies, the combination was found to be significantly less effective than daptomycin alone. This is in contrast to the results of Sakoulas et al. (32), who found that in a rat model of left-sided MRSA endocarditis, daptomycin plus rifampin significantly reduced bacterial counts in vegetations to a greater extent than did daptomycin monotherapy. The rats were treated with daptomycin doses meant to simulate the pharmacokinetics achieved with human doses, but the rifampin dose was given only once daily and the resulting pharmacokinetics were not correlated with those seen in humans, as was done in our trial. Thus, the conflicting results of these two studies may be related to the different rifampin pharmacokinetics achieved, as well as the fact that different animals and bacterial strains were employed.

Fowler et al. (10) found an increase in the MIC of daptomycin in some patients with microbiological failure in the treatment of *S. aureus* bacteremia and endocarditis. This has also been described in several case reports of patients treated with daptomycin for severe, deep-seated *S. aureus* infections (16, 26, 33, 36). In the present study, daptomycin susceptibility was reduced in a single isolate obtained from the daptomycin-only arm. Population analysis revealed heteroresistance to daptomycin in this isolate. However, all bacteria harvested from vegetations in the combination arms remained susceptible to daptomycin, gentamicin, and rifampin after 48 h of treatment. This concurs with the results of Rose et al. (31), who noted that the addition of gentamicin or rifampin prevented further increases in daptomycin MICs among susceptible and nonsusceptible strains of *S. aureus* after exposure to standard- or high-dose daptomycin in an in vitro pharmacodynamic model with simulated endocardial vegetations.

In conclusion, using a humanized pharmacokinetic model, the addition of gentamicin or rifampin does not enhance the effectiveness of daptomycin in the treatment of experimental aortic valve endocarditis due to MRSA. Further study will be required to determine if daptomycin combination therapy can be used to improve treatment outcomes or prevent the development of resistance during therapy for this type of infection.

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