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Daptomycin–β-Lactam Combinations in a Rabbit Model of Daptomycin-Nonsusceptible Methicillin-Resistant *Staphylococcus aureus* Endocarditis

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Beta-lactams enhance the *in vitro* activity of daptomycin against methicillin-resistant strains of *Staphylococcus aureus*. Experiments were performed in a rabbit model of aortic valve endocarditis caused by methicillin-resistant daptomycin-nonsusceptible *S. aureus* strain CB5054 to determine if a cephalosporin, ceftriaxone, administered as a once-daily dose of 100 mg/kg of body weight, or a carbapenem, ertapenem, administered as a once-daily dose of 40 mg/kg, improved the efficacy of daptomycin, administered as a once-daily dose of 12 mg/kg. Daptomycin was ineffective alone in reducing organism densities compared to untreated controls in vegetations and spleen, but densities were 1.4 $\log_{10}$ CFU/g lower in kidney. The combination of daptomycin plus ceftriaxone or daptomycin plus ertapenem reduced bacterial densities in all tissues compared to single agents, with 0.6 to 1.0 $\log_{10}$ CFU/g fewer organisms in vegetations, 1.5 to 2.5 $\log_{10}$ CFU/g fewer organisms in spleen, and 1.8 to 2.5 $\log_{10}$ CFU/g fewer organisms in kidney, although differences were statistically significant only in spleen for daptomycin plus ceftriaxone and in kidney for daptomycin plus ertapenem. Drug exposures in rabbits were less than those achievable in humans, which may have limited the *in vivo* activity, particularly in vegetations.

The combination of daptomycin and a beta-lactam enhances killing against daptomycin-susceptible and daptomycin-nonsusceptible methicillin-resistant *Staphylococcus aureus* (MRSA) (1–3), increases daptomycin binding to the bacterial cell membrane (2, 4, 5), and prevents the development of daptomycin resistance (3, 6, 7). Yang et al. (8) found that daptomycin plus oxacillin yielded a modest yet significant reduction (~2 to 3 $\log_{10}$ CFU/g versus daptomycin alone) in MRSA tissue counts compared to the single drugs in all target organs studied (vegetations, kidneys, and spleen) in a rabbit model of endocarditis caused by a daptomycin-nonsusceptible strain. Case reports describe the successful clearance of persistent bacteremia caused by MRSA strains, including strains that are nonsusceptible to daptomycin (2, 9–11).

The majority of studies have focused on daptomycin in combination with antistaphylococcal beta-lactams (oxacillin, nafcillin, or ceftaroline); a variety of beta-lactams, including cephalosporins and carbapenems, have been shown to enhance the activity of daptomycin *in vitro* (3, 7), suggesting that this may be a general property of daptomycin–beta-lactam combinations. Experiments were conducted with the rabbit model of endocarditis caused by a daptomycin-nonsusceptible strain of MRSA to quantify the effect of daptomycin in combination with a once-daily cephalosporin, ceftriaxone, or a once-daily carbapenem, ertapenem, in the rabbit model of infective endocarditis (12).

MATERIALS AND METHODS

Bacterial strain. *S. aureus* strain CB5054 (provided by Cubist Pharmaceuticals), a methicillin-resistant, daptomycin-nonsusceptible (Etest MICs of 24 μg/ml and 2 μg/ml, respectively) clinical isolate (8), was used to establish endocarditis. The inoculum was prepared by diluting a frozen stock in injectable 0.9% sodium chloride. The frozen stock was prepared from a culture grown overnight in tryptic soy broth. Cells were washed and resuspended in phosphate-buffered saline with 10% glycerol and stored at −80°C.

***In vitro* susceptibility studies.** MIC determinations were performed in triplicate using cation-adjusted Mueller-Hinton broth (CAMHB) with microdilution methods according to CLSI recommendations (13). Ca^{2+} was added to a final concentration of 50 μg/ml as recommended by the manufacturer for daptomycin testing.

Time-kill studies were performed in duplicate with a shaking-platform incubator at 200 rpm at 37°C in 10-ml volumes of CAMHB supplemented with 50 μg/ml Ca^{2+} at a starting inoculum of 10^6 CFU/ml. Samples (0.1 ml) were quantitatively cultured at 0, 6, and 24 h on blood agar, which was incubated for 48 h. Synergy was defined as a $\geq$2-$\log_{10}$ CFU/ml reduction for the antibiotic combination compared to the single antibiotic with the fewest CFU per milliliter at 24 h.

Animal model. Animals were maintained in accordance with American Association for Accreditation of Laboratory Animal Care criteria. These studies were approved by the Animal Research Committee (IACUC) of the University of California, San Francisco.

Endocarditis was established in New Zealand White rabbits weighing 2 to 3 kg according to standard methods (14). Briefly, a catheter was positioned across the aortic valve and sutured in place. Forty-eight hours later, an inoculum of ~10^7 CFU of the test strain in 1 ml of 0.9% saline was injected via the marginal ear vein.

Antibiotics were administered once daily for a total of 4 doses beginning 16 to 20 h after bacterial inoculation (day 1). Daptomycin was dosed at 12 mg/kg of body weight intravenously (i.v.), which corresponds to an area under the concentration-time curve (AUC) achieved by a human
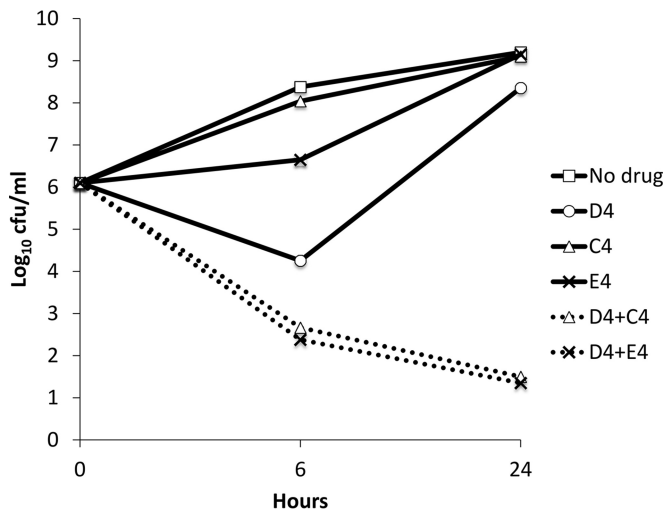
FIG 1 Time-kill studies of *Staphylococcus aureus* strain CB5054 exposed to daptomycin (D), ceftriaxone (C), or ertapenem (E) alone or daptomycin in combination with either ceftriaxone or ertapenem, each at a concentration of 4 µg/ml (averages of results from 2 separate experiments).

dose of 6 mg/ml (14); ceftriaxone was dosed at 100 mg/kg intramuscularly (i.m.); and ertapenem was dosed at 40 mg/kg i.m. A 1-ml sample of blood was obtained 1 h after dosing on day 1 or 2 and at the time of sacrifice, which was ~24 h after the last dose of antibiotic. Samples were processed and stored as plasma for measurement of drug concentrations by high-performance liquid chromatography (HPLC). Assays were performed by Cubist Pharmaceuticals.

Untreated control rabbits were sacrificed on day 1, ~16 to 20 h after bacterial inoculation. Antibiotic-treated rabbits were sacrificed on day 5, ~24 h following the fourth dose. Target tissues (entire vegetation [~0.01 to 0.05 g], kidney [0.1-g sample], and spleen [0.1-g sample]) were harvested and quantitatively cultured to determine the numbers of residual organisms. Rabbits dying before day 5 had tissues harvested and processed for culture if they received at least one dose of antibiotic and survived for at least 12 h.

Tissue samples were suspended in 1.0 ml of sterile saline and homogenized. Serial 10-fold dilutions were performed, and 0.1 ml of each dilution was plated onto blood agar. Colonies were counted after 24 to 48 h of incubation at 37°C. Tissue titers were expressed as $\log_{10}$ CFU per gram of tissue. The thresholds of detection were ~1 $\log_{10}$ CFU/g in kidney and spleen and 1.5 $\log_{10}$ CFU/g in vegetations. Tissue samples showing no growth were assigned a value corresponding to the threshold of detection.

Statistical analysis. Mean tissue titers and standard deviations (SD) were calculated for each tissue and group. Approximately 8 evaluable rabbits were required per group for 80% power and a P value of <0.05 to detect a 2-$\log_{10}$ CFU/g reduction in bacterial density for the more effective single agent compared to the antibiotic combination. A Mann-Whitney U test was used to compare the two groups, and differences for which P values were <0.05 were considered statistically significant.

RESULTS

In vitro **susceptibility studies.** MICs were 4 µg/ml, >128 µg/ml, and 16 µg/ml for daptomycin, ceftriaxone, and ertapenem, respectively. Daptomycin at 4 µg/ml, ceftriaxone at 4 µg/ml, and ertapenem at 4 µg/ml as single agents did not inhibit the growth of CB5054 in time-kill studies. The combinations of daptomycin at 4 µg/ml plus ceftriaxone at 4 µg/ml and daptomycin at 4 µg/ml plus ertapenem at 4 µg/ml were synergistic and bactericidal, with >99.9% reductions in CFU at 24 h (Fig. 1).

In vivo **studies.** Fifty-two rabbits were inoculated with strain CB5054. Five infected rabbits died prior to being allocated to control or treatment groups. Mean tissue densities ± SD in these 5 rabbits were 7.80 ± 0.49 $\log_{10}$ CFU/g in vegetations, 6.08 ± 0.58 $\log_{10}$ CFU/g in spleen, and 6.92 ± 0.51 $\log_{10}$ CFU/g in kidney, values similar to those for the untreated control group (Table 1). One daptomycin-treated rabbit that was euthanized on day 5 had no catheter across the aortic valve and no organisms isolated from any tissue cultures. This rabbit was excluded from the data analysis. The 46 remaining rabbits (6 controls and 8 in each treatment group) all had bacteria isolated from one or more tissue cultures.

Daptomycin alone generally was ineffective (Table 1). Although not statistically significant, daptomycin-treated rabbits had on average ~0.5 $\log_{10}$ CFU/g more organisms in vegetations and 0.6 $\log_{10}$ CFU/g fewer organisms in spleen tissue than did controls. Daptomycin-treated rabbits had ~1.4 $\log_{10}$ CFU/g fewer organisms in kidney tissue than did controls ($P < 0.033$).

The mean bacterial density in vegetations of rabbits treated with beta-lactams was about 1 $\log_{10}$ CFU/g lower than that in rabbits treated with daptomycin. Rabbits treated with the daptomycin-ceftriaxone combination had 1.1 $\log_{10}$ CFU/g fewer bacteria on average in vegetations than did rabbits treated with ceftriaxone alone. Rabbits treated with the daptomycin-ertapenem combination had 0.6 $\log_{10}$ CFU/g fewer bacteria in vegetations than did rabbits treated with ertapenem alone. Neither difference was statistically significantly ($P = 0.052$ and $P > 0.1$, respectively).

The mean bacterial density in spleen tissue was significantly lower, 2.6 $\log_{10}$ CFU/g, for rabbits treated with the daptomycin-ceftriaxone combination than for rabbits treated with daptomycin alone ($P = 0.014$), which was marginally more effective as a single agent than ceftriaxone. This combination achieved 1.8 $\log_{10}$ CFU/g fewer bacteria in kidney tissue than did treatment with daptomycin alone, but the difference was not statistically significant.

The bacterial density in spleen tissue was 1.5 $\log_{10}$ CFU/g lower for rabbits treated with the daptomycin-ertapenem combination than for those treated with ertapenem alone, but the difference between the combination and ertapenem alone was not statistically significant ($P = 0.066$). This combination achieved 2.4 $\log_{10}$

TABLE 1 Numbers of rabbits per treatment group and deaths and densities of organisms in tissue[a]

Treatment group	No. of rabbits (no. of deaths)	Mean density of organisms ($\log_{10}$ CFU/g) ± SD		
		Vegetation[b]	Spleen[c]	Kidney[d]
Control	6 (NA)	8.11 ± 0.67	6.53 ± 0.43	6.91 ± 0.57
Dapto	8 (4)	8.48 ± 1.52	5.95 ± 0.90	5.51 ± 1.40
Ctrx	8 (4)	8.00 ± 1.42	6.17 ± 2.19	5.57 ± 2.10
Erta	8 (6)	7.23 ± 1.64	4.99 ± 0.96	5.01 ± 1.40
Dapto + Ctrx	8 (2)	6.94 ± 1.93	3.40 ± 1.87	3.68 ± 2.39
Dapto + Erta	8 (1)	6.62 ± 1.16	3.49 ± 1.55	2.56 ± 1.37

[a] Dapto, daptomycin; Ctrx, ceftriaxone; Erta, ertapenem; NA, not applicable.
[b] $P > 0.1$ for the control group versus the daptomycin treatment group, $P = 0.052$ for the ceftriaxone group versus the daptomycin-ceftriaxone group, and $P > 0.1$ for the ertapenem group versus the daptomycin-ertapenem group.
[c] $P > 0.1$ for the control group versus the daptomycin treatment group, $P = 0.014$ for the daptomycin group versus the daptomycin-ceftriaxone group, and $P = 0.066$ for the ertapenem group versus the daptomycin-ertapenem group.
[d] $P < 0.033$ for the control group versus the daptomycin treatment group, $P > 0.1$ for the daptomycin group versus the daptomycin-ceftriaxone group, and $P = 0.010$ for the ertapenem group versus the daptomycin-ertapenem group.

TABLE 2 Plasma drug concentrations and half-lives of antibiotics used for treatment

| Antibiotic | Mean concn (µg/ml) ± SD (range) (no. of samples)[a] | | Half-life (h) |
	1 h after dosing	Trough	
Daptomycin	140 ± 28 (103–184) (7)	1.4 ± 0.6 (0.7–2.4) (8)	3.3
Ceftriaxone	300 ± 76 (209–340) (5)	2.8 ± 3.1 (0.7–8.3) (5)	3.4
Ertapenem	214 ± 40 (176–269) (4)	0.4 ± 0.2 (0.1–0.6) (4)	2.5

[a] One-hour samples were obtained on day 1 or 2. Trough samples were obtained at the time of sacrifice, ~24 h after the last dose.

CFU/g fewer bacteria in kidney tissue than did ertapenem alone ($P = 0.010$).

Survival was better for rabbits treated with daptomycin combinations than for those treated with single agents. Ten of 24 rabbits treated with single agents survived the entire 4-day course of therapy, compared to 13 of 16 treated with the combination ($P = 0.022$).

Mean serum concentration of daptomycin, ceftriaxone, and ertapenem 1 h after dosing were 140, 300, and 214 µg/ml, respectively. Half-lives were 3.3, 3.4, and 2.5 h, respectively (Table 2).

DISCUSSION

Daptomycin–beta-lactam combinations outperformed single-agent therapy in this model. Daptomycin generally was ineffective alone. Beta-lactams were numerically more effective agents than daptomycin in most instances, perhaps reflecting the heterogeneous phenotype of methicillin-resistant strains and the seesaw phenomenon described previously for daptomycin-nonsusceptible strains (8) such that higher daptomycin MICs are associated with greater susceptibility to beta-lactams. Survival was better and mean bacterial burdens were always lower in rabbits treated with the combination than in those treated with a single drug. The two combinations performed about the same.

Neither beta-lactam combination was particularly effective in reducing bacterial densities in vegetations compared to a beta-lactam as a single agent. Efficacy may have been limited by inadequate drug exposures. The 1-h plasma concentrations of antibiotics achieved in rabbits were similar to peak concentrations in humans given therapeutic doses, which are ~100 µg/ml for daptomycin at 6 mg/kg once daily, 154 µg/ml for ceftriaxone at 1 g once daily, and 150 µg/ml for ertapenem at 1 g once daily (15). However, overall drug exposures were considerably lower given that drug half-lives in rabbits were approximately half or less of the half-lives in humans, which are 8 to 9 h for daptomycin, 8 h for ceftriaxone, and 4 h for ertapenem (15). It is possible that with more frequent dosing of the beta-lactams (for example, twice daily), which would more closely simulate drug exposures in humans, and with higher doses of daptomycin (the dose administered corresponded to the standard 6-mg/kg dose in humans, which is the FDA-approved dose, although doses of 8 to 12 mg/ml are often used clinically), there would have been significantly greater reductions in bacterial densities in all tissues.

The results of these experiments with a cephalosporin and a carbapenem beta-lactam antibiotic are consistent with those of the original rabbit endocarditis studies (8), conducted with the same strain as the one used here, describing enhanced activity of daptomycin in combination with oxacillin against a daptomycin-nonsusceptible strain of MRSA. The results are also consistent

with a growing body of experimental and clinical data suggesting that combination therapy with daptomycin and a beta-lactam could improve outcomes of MRSA infections (1–3, 8, 9, 16–18). Given the high mortality rates associated with invasive staphylococcal infections, particularly those due to MRSA, clinical trials to test this hypothesis seem warranted.

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