Synergistic Activity of Ceftobiprole and Vancomycin in a Rat Model of Infective Endocarditis Caused by Methicillin-Resistant and Glycopeptide-Intermediate Staphylococcus aureus


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The therapeutic activity of ceftobiprole medocaril, the prodrug of ceftobiprole, was compared to that of vancomycin, daptomycin, and the combination of a subtherapeutic dose of ceftobiprole and vancomycin in a rat model of infective endocarditis due to methicillin-resistant Staphylococcus aureus (MRSA) (ATCC 43300) or glycopeptide-intermediate Staphylococcus aureus (GISA) (NRS4 and HIP 5836) strains. The minimum bactericidal concentrations of ceftobiprole, vancomycin, and daptomycin at bacterial cell densities similar to those encountered in the cardiac vegetation in the rat endocarditis model were 2, >64, and 8 μg/ml, respectively, for MRSA ATCC 43300 and 4, >64, and 8 μg/ml, respectively, for the GISA strain. Ceftobiprole medocaril administered in doses of 100 mg/kg of body weight given intravenously (i.v.) twice a day (BID) every 8 h (q8h) (equivalent to a human therapeutic dose of ceftobiprole [500 mg given three times a day [TID]]) was the most effective monotherapy, eradicating nearly 5 log₁₀ CFU/g MRSA or 6 log₁₀ CFU/g GISA organisms from the cardiac vegetation and had the highest incidence of sterile vegetation compared to the other monotherapies in the endocarditis model. In in vitro time-kill studies, synergistic effects were observed with ceftobiprole and vancomycin on MRSA and GISA strains, and in vivo synergy was noted with combinations of subtherapeutic doses of these agents for the same strains. Additionally, sterile vegetations were achieved in 33 and 60%, respectively, of the animals infected with MRSA ATCC 43300 or GISA NRS4 receiving ceftobiprole-vancomycin combination therapy. In summary, ceftobiprole was efficacious both as monotherapy and in combination with vancomycin in treating MRSA and GISA infections in a rat infective endocarditis model and warrants further evaluation.

Infective endocarditis (IE) remains a major health care problem today, with staphylococcal and streptococcal infections accounting for up to 90% of the cases (53). Methicillin-resistant Staphylococcus aureus (MRSA) is commonly associated with IE, especially in individuals with prosthetic cardiac valve disease and among intravenous (i.v.) drug users. Vancomycin has been used for many years to treat IE, often in combination with other antimicrobial agents (35). Daptomycin was approved in 2003 for S. aureus bloodstream infections (bacteremia), including individuals with right-sided endocarditis (57). Current guidelines for MRSA endocarditis include either vancomycin (15 to 20 mg/kg of body weight given i.v. every 12 h) or daptomycin (6 mg/kg given i.v. once daily), and the addition of rifampin plus gentamicin is indicated for prosthetic cardiac valve disease (44).

Resistance to currently marketed antibiotics continues to be a growing problem. Methicillin resistance in S. aureus limits the choice of antibiotics available to clinicians to treat staphylococcal infections. Vancomycin has become the drug of choice over the years to treat MRSA hospital infections, including endocarditis; however, it has shortcomings in terms of nephotoxicity, tissue penetration, and increasing bacterial resistance (14–16). Although glycopeptide-intermediate (27, 28, 30, 32, 43, 44, 46, 60, 62, 65) and -resistant strains (4, 8, 68) of S. aureus have been detected sporadically, it is troublesome to imagine the available treatment options that would remain if they were to become as prevalent as MRSA strains. Also, with many pharmaceutical companies reducing or eliminating programs focused on the discovery and development of antibiotics, new agents active against MRSA, glycopeptide-intermediate S. aureus (GISA), or vancomycin-resistant S. aureus (VRSA) strains may be limited in the future; thus, there is a need to consider combinations of antibiotics to find enhanced efficacy against staphylococcal infections (13).

Antibiotic synergy has been a highly debated topic among antimicrobial researchers and clinicians since the 1950s (36) with β-lactam synergy being discussed in the 1970s (18, 31). Today there are many documented cases of β-lactam synergy with vancomycin (1, 58, 63), including those observed with ceftobiprole and vancomycin (9, 17, 26, 40). Currently, there is controversy over the added benefit of antibiotic combination therapy; however, if the combination allows for a reduced dose of agents that may be associated with safety or tolerability concerns (such as, for example, vancomycin [16, 41]), or the addition of a second agent reduces the incidence of bacterial resistance, then this approach may be desirable. In support of the utility of ceftobiprole combination therapy, two studies recently reported the added therapeutic benefits of combining ceftobiprole and vancomycin in models of infective endocarditis (26, 64).

In 1997, the first documented case of MRSA with reduced susceptibility to vancomycin was reported (33), with recent studies...
associating decreased staphylococcal susceptibility to vancomycin and clinical vancomycin treatment failure, including endocarditis indications (8, 29, 59). In rabbit endocarditis, a heterogeneous vancomycin-intermediate Staphylococcus aureus (hVISA) clinical strain from an endocarditis patient resulted in vancomycin treatment failure (48). Ceftobiprole, a novel anti-MRSA cephalosporin, has been reported to be efficacious as monotherapy in the experimental IE setting, including infections due to MRSA and VISA strains (7, 24, 61, 69). Recently, in vitro synergy has been reported for ceftobiprole and vancomycin (24), and in vivo synergy for these agents has been reported for a rat model of endocarditis (23). In this study, we describe the efficacy of ceftobiprole, vancomycin, daptomycin, and the combination of ceftobiprole and vancomycin in an established rat IE model due to MRSA and GISA strains.

(Part of this work was previously presented at the 50th International Conference on Antimicrobial Agents and Chemotherapy, Boston, MA, 12 to 15 September 2010 [26].)

MATERIALS AND METHODS

Antimicrobial agents. Ceftobiprole medocaril, the prodrug of ceftobiprole, was obtained from Johnson & Johnson Pharmaceutical Research and Development, L.L.C. (Raritan, NJ). Vancomycin hydrochloride was purchased from MP Biomedicals (Irvine, CA), and daptomycin was purchased from Cubist Pharmaceuticals, Inc. (Lexington, MA). Ceftobiprole medocaril and daptomycin were prepared in sterile saline, whereas vancomycin was solubilized in sterile water.

Rat humanized dosing strategy. The ceftobiprole dose of 100 mg/kg given twice daily employed in rat studies was modeled from rat pharmacokinetic studies conducted at Johnson & Johnson (37) and published human pharmacokinetic studies (51). The schedule of 8 h between doses was optimized based on the time above the MIC (T>MIC) calculations derived from the pharmacokinetics described herein. The ceftobiprole dose of 60 mg/kg was subtherapeutic, with minimal efficacy in the rat infective endocarditis model, and was used in combination therapy to measure the effect of ceftobiprole and vancomycin in concert. Because vancomycin doses that simulate human exposure (by area under the concentration-time curve [AUC]) in the rat vary in the literature depending on the model studied (5, 19, 49), we chose doses employed in previous studies in the rat endocarditis model (120 mg/kg given twice daily) (50, 69, 70). The daptomycin dosing was adapted from the rat and human (6 mg/kg) pharmacokinetics published by Sakoulas and colleagues (56) to achieve similar AUCs between species.

Microorganisms. Staphylococcus aureus MRSA (ATCC 43300) was purchased from the American Type Culture Collection (ATCC) (Manassas, VA), and glycopeptide-intermediate S. aureus (GISA) (NRS4/HP 5836) was obtained from the Network on Antimicrobial Resistance in Staphylococcus aureus (NARS) program supported under NIAID/NIH contract HHSN27220070005C.

Animals. Female Sprague-Dawley (SD) rats (225 to 300 g) were used for all experiments. All animal studies were reviewed and approved by the Johnson & Johnson Pharmaceutical Research & Development Institutional Animal Care and Usage Committee and were conducted in accordance with the U.S. Laboratory Animal Welfare Act (7 U.S.C. 2131-2139; 7 CFR 2.22, 2.80, and 371.2). The numbers of animals were justified by a power analysis of the treatment group sample size necessary to detect a statistically significant decrease in the number of bacterial CFU by Dunnett’s multiple comparison test (66).

Inoculum preparation. Staphylococcus aureus (MRSA ATCC 43300 or GISA NR54) was grown overnight in brain heart infusion broth at 37°C with shaking at 200 rpm. The overnight culture was centrifuged, washed once with saline, diluted 1:100 in sterile saline, and adjusted to an optical density at 600 nm (OD600) of 0.132 (MRSA) or 0.234 (GISA). The adjusted culture was then further diluted 1:100 (MRSA) or 1:40 (GISA) in saline before injection. The numbers of CFU of the inoculum were determined using spiral plate methodology on tryptic soy agar (TSA).

In vitro susceptibility. Broth microdilution MIC testing followed Clinical and Laboratory Standards Institute (CLSI) methodology (11, 12) except for the use of a log-phase inoculum. In addition to the standard MIC inoculum tested (5 × 10^6 CFU/ml), increased starting inoculum concentrations of approximately 5 × 10^8, 5 × 10^7, and 5 × 10^6 CFU/ml were employed. The log-phase inoculum concentrations were confirmed by actual viable cell counts. MIC testing was performed using cation-adjusted Mueller-Hinton broth (CAMHB; TREK Sensititre, Cleveland, OH), and for daptomycin testing, CAMHB was adjusted to 50 µg/ml calcium.

The minimum bactericidal concentration (MBC) values were determined by CLSI broth microdilution methodology (52). A volume of 10 µl was plated from clear or trailing MIC wells to determine the 99.9% killing endpoint, and plate counts were read at 24 and 48 h. CLSI quality control (QC) strains S. aureus ATCC 29213 and S. aureus ATCC 25923 were evaluated concurrently for MICs and MBCs and performed within acceptable CLSI QC limits (data not shown).

Pharmacokinetics. Female SD rats were purchased with surgically implanted central venous catheters (Harlan Laboratories, Indianapolis, IN). Every 4 or 5 days until the catheters were used in the experiment, they were flushed with saline, then 20 µl of sterile lock solution (500 units/ml sodium heparin in 50% glycerol) was added, and the catheter was closed. Food and water were provided ad libitum during the experiment. At the start of the experiment, the catheters were flushed with saline to ensure patency, and then the rats were given a single intravenous dose of ceftobiprole medocaril via the tail vein. At 0.25, 0.5, 1, 2, 3, 4, 6, 8, and 24 h, the catheter was opened, 0.1 ml of blood was removed and discarded, and then 0.4 ml of blood was taken and placed into a lithium heparin tube on ice. After each time point, 0.4 ml of saline was given to the rat via the catheter, 50 µl of lock solution was added, and the catheter was closed. All time points were collected from each animal, and there were three animals in each group. The blood samples were centrifuged for 5 min at 13,000 rpm to separate the plasma. The concentrations of ceftobiprole in plasma were determined by high-performance liquid chromatography (HPLC) methodology using an Agilent 1100 HPLC system with diode array detection. To remove plasma proteins from the samples prior to analysis, plasma samples were diluted 20% with acetonitrile (vol/vol) and 20% with a 3 M solution of trichloracetic acid, vortexed, and centrifuged. The limit of detection was 0.2 µg/ml.

In vitro synergy studies. (i) Checkerboard synergy testing. Broth microdilution checkerboard synergy testing was performed by standard methodology (47). The checkerboard panel was prepared using ceftobiprole at concentrations of 0.008 to 8 µg/ml (MRSA and GISA strains) and vancomycin at concentrations of 0.06 to 4 µg/ml for MRSA and 0.25 to 16 µg/ml for GISA strains. The assay used a log-phase inoculum adjusted to a test inoculum of 5 × 10^5 CFU/ml, which was confirmed by viable CFU counts. The assay plates were incubated at 35°C for 24 h. Wells with single agents were read as MICs; ceftobiprole MIC was read at 18 h and rechecked at 24 h. The MIC was the lowest concentration of antimicrobial agent showing inhibition of visible growth. Wells with combinations of drugs were read as growth or no growth, and the lowest concentration of no visible growth was noted for each row. The fractional inhibitory concentration (FIC) of each agent was determined, and the summation of FIC concentrations of approximately 5 × 10^7, and 5 × 10^8 CFU/ml was calculated from the pharmacokinetics described herein. The ceftobiprole dose of 100 mg/kg given twice daily employed in rat studies was modeled from rat pharmacokinetic studies conducted at Johnson & Johnson (37) and published human pharmacokinetic studies (51). The schedule of 8 h between doses was optimized based on the time above the MIC (T>MIC) calculations derived from the pharmacokinetics described herein. The ceftobiprole dose of 60 mg/kg was subtherapeutic, with minimal efficacy in the rat infective endocarditis model, and was used in combination therapy to measure the effect of ceftobiprole and vancomycin in concert. Because vancomycin doses that simulate human exposure (by area under the concentration-time curve [AUC]) in the rat vary in the literature depending on the model studied (5, 19, 49), we chose doses employed in previous studies in the rat endocarditis model (120 mg/kg given twice daily) (50, 69, 70). The daptomycin dosing was adapted from the rat and human (6 mg/kg) pharmacokinetics published by Sakoulas and colleagues (56) to achieve similar AUCs between species.

(i) Time-kill studies. Suspensions prepared from overnight cultures grown on Mueller-Hinton agar were diluted to a starting inoculum of 1 × 10^6 or 5 × 10^6 in 5 ml of CAMHB for the MRSA and GISA strains, respectively. Drug concentrations for ceftobiprole in time-kill studies were based on achievable free-drug plasma levels (maximum concentration of drug in serum Cmax, 1/4 Cmax, 1/16 Cmax, 1/64 Cmax and 1/256 Cmax) determined in rat pharmacokinetic analyses for the dose (60 mg/kg) used in the endocarditis assay (22). Vancomycin concentrations were determined using spiral plate methodology on tryptic soy agar (TSA).
TABLE 1 MIC and MBC values for ceftobiprole, vancomycin, and daptomycin at different inocula against MRSA ATCC 43300 or GISA NRS4

<table>
<thead>
<tr>
<th>Strain</th>
<th>Inoculum</th>
<th>MIC/MBC (µg/ml)</th>
<th>Cefitobiprole</th>
<th>Vancomycin</th>
<th>Daptomycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSA ATCC 43300</td>
<td>4.9 × 10^5</td>
<td>1/1</td>
<td>1/1</td>
<td>0.25/0.25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.4 × 10^6</td>
<td>1/1</td>
<td>2/2</td>
<td>0.5/0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.0 × 10^7</td>
<td>1/2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4/4</td>
<td>2/2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.4 × 10^8</td>
<td>1/2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&gt;8/4/8 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>4/8</td>
<td></td>
</tr>
<tr>
<td>GISA NRS4</td>
<td>5.5 × 10^6</td>
<td>1/1</td>
<td>4/4</td>
<td>1/1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.3 × 10^6</td>
<td>1/1</td>
<td>8/8</td>
<td>2/2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.1 × 10^7</td>
<td>2/4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8/8</td>
<td>4/4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.0 × 10^8</td>
<td>2/4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&gt;64/4/4 &gt;64</td>
<td>8/8</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> A paradoxical or "Eagle effect" was observed during MIC/MBC testing.

Statistical analysis was performed using nonparametric methods. These methods included a Kruskal-Wallis test to assess the significance of differences among all treatment groups, Dunn’s multiple comparison method for evaluation of significant differences between treatment groups and control, Wilcoxon exact test for the evaluation of select pairs of treatment groups, and the Jonckheere-Terpstra test for a dose-response trend test (34). Additionally, a comparison between additive treatment effects and combination treatment effects was performed using the Wilcoxon test. Statistical differences were considered significant at the 0.05 level.

**RESULTS**

**In vitro susceptibility.** The MIC and MBC values determined for ceftobiprole and comparators are listed in Table 1 for MRSA ATCC 43300 and GISA NRS4. Using a standard inoculum (~5 × 10^8 CFU/ml), the MICs for ceftobiprole, vancomycin, and daptomycin were 1, 1, and 0.25 µg/ml, respectively. Increasing the inoculum to 10^9 CFU/ml had no effect on the ceftobiprole MIC; however, the MICs of vancomycin and daptomycin increased by 8- and 16-fold, respectively. Against the GISA strain, the MIC for ceftobiprole at the standard inoculum was also 1 µg/ml; however, the MICs for vancomycin and daptomycin both increased 4-fold over that for the MRSA strain. Increasing the inoculum of the GISA strain to 3 × 10^9 CFU/ml resulted in MICs of 2, >64, and 8 µg/ml for ceftobiprole, vancomycin, and daptomycin, respectively. Against the MRSA and GISA strains at the higher inoculum, ceftobiprole was bactericidal at 2 and 4 µg/ml, respectively; however, vancomycin and daptomycin MBCs were >64 and 8 µg/ml for both strains.

**Pharmacokinetics.** The plasma pharmacokinetics for ceftobiprole in the rat are shown in Fig. 1. Dose proportionate concentrations of ceftobiprole were observed in plasma in rats following a single intravenous dose of either 60 or 100 mg/kg, consistent with the determination of C<sub>max</sub> of 149 or 228 mg/ml, respectively. The values for time above the MIC for free drug in plasma (ft>MIC) for 60- and 100-mg/kg doses of ceftobiprole with either strain, estimated from Fig. 1, were 5.3 and >16 h, respectively.

**In vitro synergy studies.** (i) Checkerboard synergy testing. The broth microdilution checkerboard data for ceftobiprole and vancomycin are listed in Table S1 in the supplemental material. Checkerboard testing of ceftobiprole and vancomycin combinations resulted in Σ FIC values ranging from 1.06 to 1.25 and 0.75 to

**FIG 1** Plasma concentrations of ceftobiprole in the rat following a single intravenous bolus dose of ceftobiprole medocaril. Each dose was given to three animals.
was administered subcutaneously once daily.

Ceftobiprole (1/256 C\text{max}) and vancomycin (1/64 C\text{max}) (MRSA); 1/16 C\text{max} (0.6 \times \text{MIC} [GISA]) either alone or in combination. A starting inoculum of $1 \times 10^6$ or $5 \times 10^6$ in 5 ml of CAMHB was used for the MRSA or GISA strain, respectively. Each experiment was performed in duplicate on separate days.

1.13 for the MRSA and GISA strains, respectively, resulting in interpretations of indifference for both strains.

(ii) Time-kill experiments. In vitro time-kill data for ceftobiprole and vancomycin against MRSA ATCC 43300 are found in Fig. 2A and Table S2 in the supplemental material. With this MRSA isolate, $C_{\text{max}}$ (60 mg/kg dose; 149 $\mu$g/ml), 1/4 $C_{\text{max}}$ (37 $\mu$g/ml), 1/16 $C_{\text{max}}$ (9.3 $\mu$g/ml), and 1/64 $C_{\text{max}}$ (2.3 $\mu$g/ml) concentrations of ceftobiprole yielded a bactericidal effect after 24 h, whereas only the $C_{\text{max}}$ (40 $\mu$g/ml), 1/4 $C_{\text{max}}$ (10 $\mu$g/ml), and 1/16 $C_{\text{max}}$ (2 $\mu$g/ml) of concentrations of vancomycin displayed similar effects. When the 1/256 $C_{\text{max}}$ concentration of ceftobiprole (0.6 $\mu$g/ml) was combined with a vancomycin concentration of 1/64 $C_{\text{max}}$ (0.6 $\mu$g/ml), a synergistic effect ($-5.2 \log_{10}$ CFU/ml from growth control) was observed at 24 h.

Against the glycopeptide-intermediate strain (GISA NRS4), ceftobiprole remained bactericidal at concentrations corresponding to 1/4, 1/16, and 1/64 of the $C_{\text{max}}$ (Fig. 2B; see Table S3 in the supplemental material). Vancomycin was less potent against the GISA strain compared to the MRSA strain with only the 1/4 $C_{\text{max}}$ (10 $\mu$g/ml) concentration achieving a bactericidal effect (Tables S2 and S3), which was concordant with its higher MIC. A synergistic effect was noted when ceftobiprole (1/256 $C_{\text{max}}$ [0.6 $\mu$g/ml]) was paired with vancomycin (1/16 $C_{\text{max}}$ [2.5 $\mu$g/ml]) resulting in a $2.3 \log_{10}$ CFU/ml reduction of GISA organisms at the end of the 24-h testing period. The concentrations for both agents were below the MIC for the GISA strain.

**Infective endocarditis.** The efficacies of ceftobiprole, vancomycin, and daptomycin, and the combination of a subtherapeutic dose of ceftobiprole and vancomycin were evaluated in rats with infective endocarditis due to MRSA ATCC 43300 (Table 2 and Fig. 3A). In control animals infected with $5.0 \log_{10}$ CFU/rat of this MRSA strain, CFU in cardiac vegetations grew to 8.7 $\log_{10}$ per g of tissue within 24 h. Systemic exposure of MRSA reached 5.7 and 5.4 $\log_{10}$ CFU/g in the spleen and kidney, respectively. In contrast, treatment of rats for 3 days with ceftobiprole (100 mg/kg given i.v. twice a day[BID] every 8 h [q8h]), daptomycin, and vancomycin...
Efficacy of ceftobiprole (black bar, 100 mg/kg; white bar, 60 mg/kg), vancomycin (gray bar, 120 mg/kg), daptomycin (hatched bar, 50 mg/kg), or the combination of the 60 mg/kg dose of ceftobiprole and vancomycin (stippled bar) on the reduction of bacterial load in cardiac vegetation in a rat infected with MRSA ATCC 43300 (A) or a glycopeptide–intermediate strain of S. aureus (GISA), NRS4 (B), in endocarditis model. The number of animals in drug-treated groups ranged from 6 to 20 animals. Each dose was tested at least twice on separate days. Statistical significance is indicated as follows: *, P < 0.05 compared to the control value by Dunn’s test; **, P < 0.05 compared to the vancomycin value by the Wilcoxon two-sample test; ***, P < 0.05 compared to the values for animals given ceftobiprole (60 mg/kg) plus vancomycin (120 mg/kg) (additive effects) by the Wilcoxon two-sample test.

FIG 3 Efficacy of ceftobiprole (black bar, 100 mg/kg; white bar, 60 mg/kg), vancomycin (gray bar, 120 mg/kg), daptomycin (hatched bar, 50 mg/kg), or the combination of the 60 mg/kg dose of ceftobiprole and vancomycin (stippled bar) on the reduction of bacterial load in cardiac vegetation in a rat infected with MRSA ATCC 43300 (A) or a glycopeptide–intermediate strain of S. aureus (GISA), NRS4 (B), in endocarditis model. The number of animals in drug-treated groups ranged from 6 to 20 animals. Each dose was tested at least twice on separate days. Statistical significance is indicated as follows: *, P < 0.05 compared to the control value by Dunn’s test; **, P < 0.05 compared to the vancomycin value by the Wilcoxon two-sample test; ***, P < 0.05 compared to the values for animals given ceftobiprole (60 mg/kg) plus vancomycin (120 mg/kg) (additive effects) by the Wilcoxon two-sample test.

resulted in reductions in the cardiac vegetation of 4.8, 3.8, and 1.2 log_{10} CFU/g of tissue, respectively, compared to control animals at the start of the treatment period. In addition to being statistically different from control animals (P < 0.05), cardiac vegetations in animals treated with ceftobiprole at 100 mg/kg and daptomycin each were statistically different (P < 0.001) from animals receiving vancomycin monotherapy. Sterile vegetations were achieved in 47% and 19% of the animals receiving ceftobiprole (100 mg/kg) and daptomycin monotherapy, respectively. Animals treated with a lower dose of ceftobiprole (60 mg/kg given i.v. BID q8h) had a 0.7 log_{10} reduction of CFU/g of cardiac vegetation compared to control animals. In contrast, animals treated for 3 days with the combination of a lower dose of ceftobiprole and vancomycin had a 4.7 log_{10} CFU/g decrease in cardiac vegetations compared to control animals and were significantly different (P < 0.005) from the additive effects of the two monotherapies. In the group receiving the combination of vancomycin and a lower dose of ceftobiprole, 33% of the animals had sterile cardiac vegetations. All drugs, including the lower dose of ceftobiprole, showed significant (P < 0.05) systemic (spleen and kidney) reductions of MRSA ATCC 43300.

After the animals were treated for 3 days, a separate group of animals was provided a three-day period without drug (relapse) prior to evaluation of bacterial load in the tissues. Rats in the ceftobiprole (100 mg/kg, i.v., BID, q8h), daptomycin, and vancomycin groups had reductions in the numbers of MRSA-infected cardiac vegetations by 2.8 (P < 0.05), 1.1, and 0.7 log_{10} CFU/g of tissue, respectively, compared to control animals at the start of the treatment period (Table 2 and Fig. 3A). Also the group receiving the combination of vancomycin and a lower dose of ceftobiprole had a 3.2 log_{10} CFU/g decrease of MRSA in the cardiac vegetation (P < 0.05) during the relapse phase. Sterile vegetations were noted in 22% or 11% of the animals receiving ceftobiprole (100 mg/kg) or the ceftobiprole–vancomycin combination therapy, respectively.

The efficacy of ceftobiprole, vancomycin, daptomycin, and the combination of ceftobiprole (60 mg/kg) and vancomycin in rats with infective endocarditis due to an S. aureus strain with reduced susceptibility to vancomycin (GISA, NRS4) is listed in Table 3 and Fig. 3B. Similar to the MRSA strain, the bacterial loads in the cardiac vegetations, spleens, and kidneys of control animals were 8.8, 5.5, and 5.2 log_{10} CFU/g of tissue, respectively; however, the infecting inoculum at 6.0 log_{10} CFU/rat was 1.0 log_{10} CFU higher than the MRSA strain. Compared to untreated controls, CFU decreases of the GISA strain in the cardiac vegetation in animals treated with ceftobiprole (100 mg/kg) and the combination of vancomycin and a subtherapeutic dose of ceftobiprole were 5.6 and 5.8 log_{10} CFU/g of tissue, respectively (P < 0.05). Sterile cardiac vegetations were noted in 60% of both of these groups. Daptomycin treatment resulted in a 4.5 log_{10} CFU/g decrease of the GISA strain from the cardiac vegetation (20% sterile), while treatment with the monotherapies of vancomycin and subtherapeutic ceftobiprole (60 mg/kg) resulted in decreases of only 1.2 and 0.9 log_{10} CFU/g, respectively.

During the relapse period, rats in the ceftobiprole (100 mg/kg, i.v., BID, q8h) and daptomycin groups had reductions in the GISA-infected cardiac vegetations by 4.8 and 4.2 log_{10} CFU/g of tissue (P < 0.05), respectively, compared to control animals at the start of the treatment period (Table 3 and Fig. 3B). Vancomycin-treated animals showed a 0.2 log_{10} CFU/g increase of the GISA strain compared to vancomycin monotherapy (20% sterile), while treatment with the monotherapies of vancomycin and subtherapeutic ceftobiprole (60 mg/kg) resulted in decreases of only 1.2 and 0.9 log_{10} CFU/g, respectively.

When cardiac vegetation samples were grown on Mueller-Hinton agar containing 4X MIC of the associated treatment antibiotic, no resistance emergence on therapy was apparent in the treatment or relapse phases of the study.

The incidences of all-cause mortality for all agents are listed in Table 4. In general, mortality was low (0 to 10%) for either strain (MRSA ATCC 43300 or GISA NRS4) in the ceftobiprole (100 mg/kg) group, the vancomycin group, or the combination group of ceftobiprole (60 mg/kg) and vancomycin during the treatment or relapse phases of the study. In contrast, when the subtherapeutic dose of ceftobiprole (60 mg/kg) was given as monotherapy, mortality tended to be slightly higher (14 to 33%, MRSA; 10 to 17% GISA), similar to what was noted in daptomycin-treated animals (9 to 27%).
In this study, we describe the efficacy of ceftobiprole monotherapy and the synergy of ceftobiprole and vancomycin in a rat endocarditis model with two clinical isolates of MRSA with different vancomycin susceptibilities. Ceftobiprole at 100 mg/kg, the dose that models a similar AUC exposure to the dose used in phase III human clinical trials (500 mg given three times a day [TID]) (37, 51), was the most effective monotherapy in eradicating MRSA ATCC 43300 or the GISA strain from the cardiac vegetation during the efficacy or relapse phase of the study. At this ceftobiprole dose (100 mg/kg), the plasma fT>MIC for either strain was greater than 66% of the dosing interval, consistent with published staphylococcal fT>MIC targets for this pharmacodynamic parameter in animals (45, 51). Also, the highest incidence of sterile vegetations was noted with the ceftobiprole dose of 100 mg/kg compared to the other monotherapies with either strain. While the number of animals having sterile cardiac vegetations was reduced somewhat during the relapse phase of the studies (MRSA and GISA strains), it is noteworthy to mention that 22% and 50% of the animals receiving ceftobiprole (100 mg/kg) in the MRSA and GISA experiments, respectively, still had sterile vegetations following tissue evaluations performed 3 days after cessation of drug treatment.

Although vancomycin remains the drug of choice to treat many MRSA-infected patients in hospitals, its clinical utility can be limited by toxicity, poor tissue penetration, slow bactericidal effect, or infections involving strains with reduced glycopeptide susceptibilities. Hence, there is a need to either reduce the clinical dose of vancomycin or enhance its efficacy against more difficult-to-treat isolates or both (14–16). In other models of infective endocarditis, treatment with vancomycin has been shown to be minimally effective (69, 70). Last year, we reported that the combination of ceftobiprole and vancomycin was more efficacious than the individual monotherapies against MRSA (26). Also, work by Entenza and colleagues showed that the ceftobiprole-vancomycin combination acted synergistically in time-kill studies (24) as well as in a rat endocarditis model due to vancomycin-intermediate S. aureus (VISA) strains (25). Our data in this report indicate that the combination of a subtherapeutic dose of ceftobiprole and vancomycin exhibits synergy (P < 0.05) in reducing bacterial load due to vancomycin-susceptible and -intermediate strains (P < 0.05) in cardiac vegetations.

### DISCUSSION

### TABLE 3 Efficacy of ceftobiprole, vancomycin, and combination therapy on target tissues in a rat model of infective endocarditis due to GISA NRS4

<table>
<thead>
<tr>
<th>Phase and antibiotic treatment</th>
<th>Dose (mg/kg)</th>
<th>No. of animals</th>
<th>Cardiac vegetation (％ sterile)</th>
<th>Spleen</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (control)</td>
<td></td>
<td>14</td>
<td>8.75 ± 0.6</td>
<td>5.47 ± 0.6</td>
<td>5.21 ± 0.8</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceftobiprole</td>
<td>100</td>
<td>10</td>
<td>3.14 ± 1.3 (60)(^{b,c})</td>
<td>2.41 ± 0.3(^{b,c})</td>
<td>2.48 ± 0.5(^{b,c})</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>12</td>
<td>7.88 ± 1.4 (0)</td>
<td>3.27 ± 1.1(^{b,c})</td>
<td>2.66 ± 0.5(^{b,c})</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>120</td>
<td>15</td>
<td>7.50 ± 1.0 (0)</td>
<td>5.19 ± 0.8</td>
<td>4.32 ± 1.1</td>
</tr>
<tr>
<td>Daptomycin</td>
<td>50</td>
<td>10</td>
<td>4.29 ± 2.0 (20)(^{b,c})</td>
<td>2.88 ± 1.2(^{b,c})</td>
<td>2.84 ± 0.9(^{b,c})</td>
</tr>
<tr>
<td>Ceftobiprole + vancomycin</td>
<td>60 + 120</td>
<td>10</td>
<td>2.90 ± 1.1 (60)(^{b,d})</td>
<td>2.30 ± 0(^{b,d})</td>
<td>2.30 ± 0(^{b,d})</td>
</tr>
<tr>
<td>Relapse</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceftobiprole</td>
<td>100</td>
<td>10</td>
<td>3.99 ± 2.3 (50)(^{b,c})</td>
<td>2.90 ± 1.1(^{b,c})</td>
<td>2.59 ± 0.9(^{b,c})</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>10</td>
<td>5.77 ± 2.5 (30)(^{b,c})</td>
<td>3.07 ± 1.2(^{b,c})</td>
<td>2.70 ± 0.7(^{b,c})</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>120</td>
<td>11</td>
<td>8.93 ± 1.1 (0)</td>
<td>5.64 ± 0.4</td>
<td>4.84 ± 0.8</td>
</tr>
<tr>
<td>Daptomycin</td>
<td>50</td>
<td>11</td>
<td>4.56 ± 2.8 (45)(^{b,c})</td>
<td>3.51 ± 1.6(^{b,c})</td>
<td>2.84 ± 0.7(^{b,c})</td>
</tr>
<tr>
<td>Ceftobiprole + vancomycin</td>
<td>60 + 120</td>
<td>10</td>
<td>2.30 ± 0 (100)(^{b,d})</td>
<td>2.30 ± 0(^{b,d})</td>
<td>2.30 ± 0(^{b,d})</td>
</tr>
</tbody>
</table>

\(^{a}\) Ceftobiprole was administered intravenously twice daily separated by 8 h for 3 days, vancomycin was administered subcutaneously twice daily separated by 8 h, and daptomycin was administered subcutaneously once daily.

\(^{b}\) P < 0.05 compared to the control value by Dunn’s test.

\(^{c}\) P < 0.001 compared to the vancomycin value by the Wilcoxon two-sample test.

\(^{d}\) P < 0.005 compared to the values for animals given ceftobiprole (60 mg/kg BID) plus vancomycin (120 mg/kg BID) (additive effect) by the Wilcoxon two-sample test.

### TABLE 4 All-cause mortality during treatment in the rat endocarditis study

<table>
<thead>
<tr>
<th>Antibiotic treatment</th>
<th>Dose (mg/kg)</th>
<th>Route(^{a})</th>
<th>Incidence of mortality (no. of dead rats/total no. of rats [%])</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>MRSA ATCC 43300</td>
</tr>
<tr>
<td>Ceftobiprole</td>
<td>100</td>
<td>i.v.</td>
<td>0/15 (0)</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>i.v.</td>
<td>5/15 (33)</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>120</td>
<td>i.v.</td>
<td>1/21 (5)</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>s.c.</td>
<td>3/11 (27)</td>
</tr>
<tr>
<td>Ceftobiprole + vancomycin</td>
<td>60 + 120</td>
<td>i. v. + s.c.</td>
<td>1/13 (8)</td>
</tr>
</tbody>
</table>

\(^{a}\) i.v., intravenous; s.c., subcutaneous.
Synergy against both strains was noted in the efficacy and relapse arms of the studies with tissues being evaluated 1 and 4 days postantibiotic therapy, respectively. Also noteworthy was the incidence of sterile vegetations in 60% and 100% of the animals receiving combination therapy during the efficacy and relapse phases of the GISA study.

Because there is some controversy over the definition of the endpoint for in vivo synergy (6, 25), we chose the most conservative definition wherein the efficacy of the combination group needed to be greater than the additive effects of the two monotherapies at the same dose used in the combination. By these criteria, synergy for the combination of ceftobiprole and vancomycin was observed (P < 0.05) in our studies for both the MRSA and GISA strains (CFU reductions of approximately 2.8 and 3.7 log_{10} CFU, respectively), supporting the potential of this combination for clinical utility.

The in vitro data in this report support our in vivo findings as the MICs/MBCs for ceftobiprole were only slightly affected by higher inocula (Table 1) which correlate with its enhanced in vivo efficacy compared to vancomycin against the MRSA strain. Vancomycin potency was severely altered by increasing the inocula. In fact, when vancomycin was tested at inocula near the concentration seen in cardiac vegetations (10^8 to 10^9 CFU/ml), the MBC rose to >64 μg/ml for both strains tested. The MBCs of ceftobiprole were also affected but to a lesser extent (8 μg/ml). These observations are concordant with the references in the literature of vancomycin being highly susceptible to increased inocula and daptomycin being affected to a lesser extent (15, 39, 55). A paradoxical effect resulting in the decreased killing of bacteria with increasing concentrations of penicillin was first observed by Eagle and Musselman in 1948 (20, 21). Since then, the in vitro “Eagle effect” has been reported in the literature for many antimicrobial agents, including ceftobiprole (2, 3, 17, 54). Interestingly, in our studies, at the higher inocula tested (10^7 and 10^8 CFU/ml) for both MIC and MBC, ceftobiprole displayed the paradoxical, or “Eagle effect” with growth of either strain occurring at concentrations ≥4-fold above the MIC (data not shown). For S. aureus, the paradoxical effects are usually ignored (52), as was the case for reporting ceftobiprole results.

In this report, we also characterize the synergy between ceftobiprole and vancomycin against an MRSA strain and a GISA strain using in vitro checkerboard and time-kill methods. Ceftobiprole has been shown to be bactericidal at 0.5× to 4× MIC in time-kill studies for many MRSA strains, including hospital-associated (HA)- and community-associated (CA) MRSA (40, 42), and a 5 μg/ml concentration (10× MIC) of ceftobiprole was shown to be bactericidal at 24 h for an MRSA strain and the same GISA strain that was used in our studies (7). Similarly, we observed bactericidal activity with ceftobiprole at ≥2× the MIC against the MRSA and GISA strains with the time-kill studies. Additionally, when human clinical exposures of ceftobiprole were simulated in an in vitro pharmacodynamic model (T>MIC of ≥100%) against the GISA NRS4, ceftobiprole was bactericidal (71). Our in vitro time-kill studies with this strain also showed similar results. Although the combinations of ceftobiprole and vancomycin were interpreted as indifferent when tested by broth microdilution checkerboard methods within this report, synergy between ceftobiprole and vancomycin was noted with time-kill methodology and in the in vivo model. Notably, the correlation between checkerboard and time-kill methodologies in assessing synergy has been observed to be variable and inconsistent for a number of different classes of antibiotics (67). More importantly, we characterized consistent synergy between ceftobiprole and vancomycin by the in vitro time-kill method and also in the rat endocarditis model.

In summary, ceftobiprole was highly effective as monotherapy in a rat IE model of staphylococcal endocarditis and displayed in vitro and in vivo synergy in combination with vancomycin in MRSA or GISA clinical strains. The combination of ceftobiprole with vancomycin at appropriate clinical doses warrants further preclinical exploration in this setting and other staphylococcal infection settings wherein monotherapy may limit clinical outcomes achievable with currently approved agents.

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REFERENCES


