Comparative Evaluation of Daptomycin (LY146032) and Vancomycin in the Treatment of Experimental Methicillin-Resistant Staphylococcus aureus Osteomyelitis in Rabbits

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A rabbit model for methicillin-resistant Staphylococcus aureus (MRSA) osteomyelitis was used to compare treatment with daptomycin, a new peptolide, and vancomycin. Daptomycin (4 mg/kg) and vancomycin (40 mg/kg) were injected subcutaneously every 12 and 6 h, respectively. After treatment, MRSA was found in bone cultures from 18 of 18 control rabbits, 10 of 17 animals treated with daptomycin, and 11 of 18 animals treated with vancomycin. Drug concentrations were measured in serum, uninfected bone, and infected bone 1 h after daptomycin or vancomycin was injected in a group of rabbits that had been infected for 3 to 4 weeks. Vancomycin was present at the highest concentrations in infected and uninfected bone. The results of this study suggest that daptomycin was similar to vancomycin in the eradication of MRSA from infected bone in an experimental model of osteomyelitis.

Methicillin-resistant Staphylococcus aureus (MRSA) causes a variety of infections, including osteomyelitis (8–11, 16–18). Heretofore, vancomycin has been the only reliable antibiotic available for treating MRSA infections (16, 17). However, long-term vancomycin therapy, especially in combination with the aminoglycosides, may cause nephrotoxicity and ototoxicity (16). In addition, MRSA strains may ultimately develop resistance to vancomycin. Thus, it is essential to have other effective and safe antibiotics to eradicate this organism.

Daptomycin, a new peptolide developed by Eli Lilly & Co., Indianapolis, Ind., exhibits in vitro inhibitory and bactericidal activities against a wide spectrum of gram-positive bacteria, including MRSA (5, 7). Daptomycin acts mainly by inhibiting cell wall synthesis and disrupting membrane permeability (4). To compare the efficacy of daptomycin and vancomycin in the treatment of MRSA osteomyelitis, we performed a controlled animal study. In an effort to reduce the variables of a clinical antibiotic study, we modified the rabbit model for MRSA osteomyelitis from the model developed by Norden and Kennedy (14).

MATERIALS AND METHODS

Induction of osteomyelitis infection. The MRSA strain used in the study was obtained from an osteomyelitis patient undergoing treatment at The University of Texas Medical Branch, Galveston. The organism was stored at −70°C in defibrinated sheep blood until needed. Antibiotic tube dilution susceptibilities (6) of this MRSA strain (10⁵ CFU/ml) to daptomycin and vancomycin were measured in cation-supplemented Mueller-Hinton broth. Daptomycin had an MIC of 0.39 μg/ml and an MBC of 0.39 μg/ml. The corresponding values for vancomycin were 0.78 and 3.13 μg/ml, respectively.

New Zealand White female rabbits, 8 weeks old and weighing 1.5 to 2.0 kg, were used in this study. Two days prior to infection an 18-gauge needle was inserted percutaneously through the lateral aspect of the left tibial metaphysis into the intramedullary cavity and 0.2 ml of 5% sodium morrhuate (Lilly) was injected. The needle was removed, and the animal was returned to its cage. Two days later another 18-gauge needle was inserted into the tibial metaphysis and 0.1 ml of 5% sodium morrhuate, 0.1 ml of MRSA (10⁶ CFU/ml), and 0.2 ml of sterile 0.85% saline were sequentially injected (13).

Therapeutic trials. The rabbits were randomized into three groups at the time of infection (day 0), and treatment was begun 14 days later. At least three infected but untreated controls (group 1) were included in each treatment series. Group 2 received daptomycin (4 mg/kg) every 12 h, and group 3 received vancomycin (Lilly) (40 mg/kg) every 6 h. The antibacterial agents were given from day 14 through day 42 (28 days total). Injections were administered subcutaneously into the back of the neck. Following completion of the treatment regimen, the rabbits were observed for 2 weeks before sacrifice.

Roentgenograms of both tibias were taken at antibiotic initiation (day 14), at antibiotic termination (day 42), and before sacrifice (day 56). The severity of the infection by roentgenographic appearance was graded by a rating system previously reported (13). The rabbits were weighed before infection and once weekly until they died or were sacrificed.

Bone cultures. Rabbits that died before treatment was begun at day 14 were not included in the study. At the conclusion of the study, rabbits were sacrificed by an intracardiac injection of sodium pentobarbital. Both tibias were removed, dissected free of all soft tissue, and processed for bacterial cultures. Using a 5-mm, single-action rongeur, we split the bones into small pieces and removed the marrow. The bone pieces were then pulverized in a bone mill and suspended in 10 ml of sterile 0.85% saline. The marrow was likewise placed in 10 ml of sterile 0.85% saline. Tenfold serial dilutions were made and streaked onto a tryptic soy agar blood plate to quantitate the bacterial colonies present. Tube dilution susceptibilities were determined for the MRSA colonies recovered from the bone and/or bone marrow.

Drug kinetics in serum and simultaneous measurements of...
drug levels in serum and bone. A group of uninfected animals and a group of animals that was selected 3 to 4 weeks after infection were given a single subcutaneous injection of daptomycin (4 mg/kg) or vancomycin (40 mg/kg). Concentrations of daptomycin and vancomycin in serum were determined with blood drawn at 1, 3, 6, and 12 h after injection. In another group of uninfected animals and another group of infected animals (3 to 4 weeks), simultaneous concentrations in serum and bone were determined 1 h after injection of daptomycin and vancomycin.

Drug assays in serum and bone. An agar disk diffusion bioassay was used to measure drug concentrations in both serum and bone eluates. *Sarcina lutea* (ATCC 9341) served as the test organism for daptomycin. The lower limit of detection of daptomycin in serum was 0.20 μg/ml. Standards of daptomycin and vancomycin (stored at 1,000 μg/ml at −20°C) were prepared with known concentrations of antibiotic diluted in 100% normal rabbit serum. Standards ranged from 0.20 to 100 μg/ml and were diluted by serial twofold decrements. Serum standards or serum samples (20 μl) were placed on blank concentration disk (0.25 in. [0.635 cm]; Difco), plated on the seeded agar plates, and incubated at 37°C overnight. The diameters of the zones of inhibition of growth of *B. subtilis* or *S. lutea* were measured for both samples and standards. Unknown concentrations were determined from semilogarithmic standards curves. Blood was drawn from each rabbit prior to the administration of either daptomycin or vancomycin. If inhibitors were detected the animal was not used in the study.

Infected or uninfected bone was prepared for assay as described above. The crushed bone was weighed and suspended in 50% 0.1 M sodium phosphate buffer (pH 7.5)–50% normal rabbit serum. One milliliter of the buffer-serum solution was used per 0.5 g of bone. The crushed bone was agitated in an Erlenmeyer flask for 1 h at 4°C with a magnetic stirring bar. Antibiotic in the supernatant fluid was assayed by the above-described disk diffusion method. Standard solutions of drugs were prepared by adding normal uninfected bone to the buffer-serum solution containing known amounts of drug. The lower limit of detection of the assay was 0.20 μg/ml for daptomycin and vancomycin.

Statistics. The chi-square test and Student's *t* test were used for datum analysis.

RESULTS

Of the 56 infected rabbits that were included in the study (Table 1), 3 (5.4%) died before treatment, which began on day 14, and were not included in the datum analysis. Three animals died after therapy was discontinued. The number of MRSA (mean ± standard error of the mean [SEM] log$_{10}$ in five rabbits sacrificed at day 14 [beginning of therapy]) was 4.81 ± 0.34 CFU/ml.

Bone cultures. Cultures from 18 (100%) of 18 untreated infected (control) rabbits yielded MRSA. Cultures were positive for MRSA in 10 (59%) of 17 rabbits treated with daptomycin and 11 (61%) of 18 rabbits treated with vancomycin. The difference between each drug treatment group and the corresponding control group was significant (*P* < 0.05). No significant difference was found in the proportion of rabbits with positive bone cultures between the treatment groups.

The MIC and MBC of daptomycin for this MRSA strain were 0.39 μg/ml. The MIC and MBC of vancomycin for this

| Table 1. Effects of daptomycin and vancomycin on experimental MRSA osteomyelitis |
|---------------------------------|---------------------------------|----------------|---------------|
| **Rabbits** | **CFU of S. aureus** | **No. that** | **Radiographic severity score** |
| | in bone cultures | **died/had death** | **on day 14 (μg/ml)** |
| **Control** | 18/18 (100%) | 10/10 (100%) | 10/10 (100%) |
| **Daptomycin treated** | 18/18 (100%) | 6/18 (33%) | 6/18 (33%) |
| **Vancomycin treated** | 18/18 (100%) | 10/18 (56%) | 10/18 (56%) |

*At day 14 beginning of therapy the number of MRSA (mean ± SEM log$_{10}$ was 4.81 ± 0.34 CFU/ml (*P* = 0.5).
MRSA strain were 0.78 and 3.12 μg/ml, respectively. The MICs and MBCs of the two drugs for the MRSA isolates recovered from the treated rabbits were all within one dilution of the corresponding values for the MRSA isolates used to infect the rabbits.

The number of viable bacteria (mean ± SEM log_{10}) found in the pulverized infected tibia from infected control rabbits (4.62 ± 0.26 CFU/ml) was significantly higher than those for both the daptomycin (1.89 ± 0.33 CFU/ml) and vancomycin (1.44 ± 0.22 CFU/ml)-treated groups (P < 0.001). No significant difference was found between the daptomycin- and vancomycin-treated groups.

The number of viable bacteria (mean ± SEM log_{10}) found in the marrow from infected control rabbits (4.09 ± 0.34 CFU/ml) was significantly higher than those for both the daptomycin (0.23 ± 0.17 CFU/ml)- and vancomycin (0.27 ± 0.17 CFU/ml)-treated groups (P < 0.001). No significant difference was found between the daptomycin- and vancomycin-treated groups.

Weight changes. The mean weights (Table 1) for all three groups of rabbits increased from day 0 through the end of the study (day 56). All of the groups, the rabbits receiving vancomycin gained the least amount of weight (0.76 kg) during the study (P < 0.05).

Roentgenographic severity. The roentgenographic severity scores of infected bones measured before therapy (week 2) were similar for the control group and both treatment groups (Table 1). The severity scores showed a significant improvement (P < 0.05) from weeks 2 to 8 of the study in both treatment groups. The control rabbits had nearly identical mean severity scores throughout the study. The majority of the rabbits had sequestra. At week 8, both the daptomycin- and vancomycin-treated groups had lower severity scores than did the control group (P < 0.05). Of the two treatment groups, the vancomycin group had a lower severity score than did the daptomycin group (P < 0.05).

Concentrations of antibiotics in serum and bone. Concentrations of daptomycin and vancomycin in serum after single subcutaneous injections of the respective drugs are shown in Table 2. The profiles in infected and uninfected rabbits were identical for each drug. The calculated half-lives for daptomycin and vancomycin were 7.75 and 7.50 h, respectively.

Simultaneous concentrations of daptomycin and vancomycin in serum, uninfected bone, and infected bone are shown in Fig. 1. The respective mean concentrations of the two drugs were significantly higher in infected bone than in uninfected bone (P < 0.05). No detectable level of daptomycin was found in uninfected bone.

**DISCUSSION**

MRSA has been isolated from cases of osteomyelitis (8–11, 16–18). With the increase in MRSA colonization and infection now being seen, the incidence of MRSA osteomyelitis can be expected to rise. Currently, vancomycin is the only reliable parenteral antibiotic for the treatment of MRSA infections, including osteomyelitis (16, 17). However, long-term vancomycin therapy may cause nephrotoxicity and ototoxicity, especially when combined with an aminoglycoside (16). Although vancomycin is the drug of choice for the treatment of MRSA infections, the results of treatment of experimental methicillin-susceptible S. aureus osteomyelitis have been disappointing (15). Norden and Shaffer (15) have shown that vancomycin kills poorly under anaerobic conditions and postulated that the low oxygen tensions found in osteomyelitic bone (12) decreased the effectiveness of vancomycin in experimental methicillin-susceptible S. aureus osteomyelitis. Although it is difficult to extrapolate data from methicillin-susceptible S. aureus to MRSA osteomyelitis, it is clear that new effective antibiotics are needed to treat MRSA osteomyelitis. Daptomycin, a new lipopeptide antibiotic with a long half-life, has excellent activity against MRSA (5, 7). If daptomycin proves to have activity equal to or greater than that of vancomycin against MRSA osteomyelitis and to have negligible toxicity, it will be a valuable new agent for the treatment of bone infections.

**TABLE 2. Concentrations of daptomycin and vancomycin in serum**

<table>
<thead>
<tr>
<th>Group and drug (ng/kg)*</th>
<th>Conc (μg/ml) (mean ± SEM) at:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 h</td>
</tr>
<tr>
<td>Uninfected</td>
<td></td>
</tr>
<tr>
<td>Daptomycin (4)</td>
<td>31.2 ± 1.88</td>
</tr>
<tr>
<td>Vancomycin (40)</td>
<td>28.7 ± 1.62</td>
</tr>
<tr>
<td>Infected</td>
<td></td>
</tr>
<tr>
<td>Daptomycin (4)</td>
<td>34.7 ± 1.14</td>
</tr>
<tr>
<td>Vancomycin (40)</td>
<td>32.8 ± 1.74</td>
</tr>
</tbody>
</table>

*There were six animals in each drug treatment group.
vancomycin were similarly effective in eradicating MRSA in experimental osteomyelitis. A total of 41% of daptomycin- and 39% of vancomycin-treated rabbits had negative tibial cultures for MRSA. Twenty-eight days of therapy with daptomycin or vancomycin reduced the bacterial counts in infected bone by approximately $3 \log_{10}$ CFU as compared with untreated controls. The marrow cultures from the infected control rabbits were not significantly different from the bone cultures. However, in the treated groups the quantitative marrow cultures had significantly fewer counts than did the quantitative bone cultures. Marrow presumably was exposed to higher concentrations of daptomycin and vancomycin than was bone. The MRSA isolated from the rabbits with positive tibial cultures showed no evidence of resistance to the treatment antibiotics. Although radiographic improvement was seen with both agents, there was greater improvement in the group receiving vancomycin. Since initial radiographic scores were similar for all groups (week 2), the severity of osteomyelitis was approximately comparable for all rabbits at the start of therapy.

Doses of daptomycin and vancomycin that would produce concentrations in serum which exceeded eight times the MIC for this MRSA strain were selected. Concentrations of daptomycin and vancomycin in serum were detectable for 12 h, and the serum curve for daptomycin was very similar to that for vancomycin. However, daptomycin was given every 12 h and vancomycin was given every 6 h despite similar half-lives. Thus, the dosing schedule should have favored vancomycin. Vancomycin should have achieved a higher steady-state concentration throughout the 28-day treatment period than daptomycin should have.

For each antibacterial agent, concentrations were significantly higher in infected bone than in uninfected bone. Vancomycin was present in significantly higher levels in both infected and uninfected bone than was daptomycin. Daptomycin was not detectable in uninfected bone. The concentration of vancomycin in bone as a percentage of the peak concentration in serum (6.0%) was greater than that of daptomycin (1.3%). This finding could be related to differences in serum protein binding between daptomycin and vancomycin.

In contrast to our osteomyelitis results, daptomycin has been found marginally effective in the endocarditis animal model (2, 3). The reasons are unclear; however, protein binding may play a role. It has been postulated that only the unbound portion of an antimicrobial agent is biologically active in serum and tissue. Daptomycin is 90 to 93% protein bound in humans and approximately 80% protein bound in rabbits. Thus, the high protein binding of daptomycin may be one of the reasons for the marginal activity of daptomycin in vivo despite its excellent in vitro activity for most gram-positive organisms. The amount of free daptomycin in osteomyelitic bone, likewise, should be marginal. However, increased ionized calcium levels have been shown to potentiate the peptidolites (4), including daptomycin (1). Thus, the decreased amount of free daptomycin in bone may be offset by the increased activity of this antibiotic in areas where high ionized calcium concentrations are present.

Generally, it is more difficult to cure experimental osteomyelitis in rabbits than in humans, in whom extensive debridement surgery is used. Thus, if results with the rabbit model can be extrapolated to humans, it appears that daptomycin provides therapy analogous to that of vancomycin in MRSA osteomyelitis when good MICs and MBcs are demonstrated by tube dilution testing.

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**LITERATURE CITED**


