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EXPERIMENTAL THERAPEUTICS

Exebacase in Addition to Daptomycin Is More Active than Daptomycin or Exebacase Alone in Methicillin-Resistant *Staphylococcus aureus* Osteomyelitis in Rats

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ABSTRACT Bacteriophage-derived lysins are being developed as anti-infective agents. In an acute osteomyelitis methicillin-resistant *Staphylococcus aureus* (MRSA) model, rats receiving no treatment or treatment with daptomycin, exebacase (CF-301), or daptomycin plus exebacase had means of 5.13, 4.09, 4.65, and 3.57 $\log_{10}$ CFU/gram of bone, respectively. All treated animals had fewer bacteria than did untreated animals ($P \leq 0.0001$), with daptomycin plus exebacase being more active than daptomycin ($P = 0.0042$) or exebacase ($P < 0.001$) alone.

KEYWORDS CF-301, daptomycin, exebacase, methicillin-resistant *Staphylococcus aureus*, osteomyelitis

Osteomyelitis is a devastating infection that can be challenging to treat and that may be associated with morbidity, including damage to bone tissue and metastatic infection. *Staphylococcus aureus* is the most common cause of osteomyelitis (1). *S. aureus* has the ability to form biofilms and to enter and survive within osteoblasts, both of which may allow it to evade the immune system and be resistant to many traditional antimicrobials (2). Current therapeutic options include surgical irrigation and debridement and long-term therapy with antimicrobials such as daptomycin or vancomycin (1, 3, 4). Compounding the challenge of the virulence of *S. aureus* itself is the increasing antimicrobial resistance of *S. aureus*. Methicillin-resistant *S. aureus* (MRSA) osteomyelitis is growing in frequency and has been associated with poorer outcomes than methicillin-susceptible *S. aureus* infection (5). There is therefore a need for new, more effective antimicrobial strategies for *S. aureus*, especially MRSA, bone and joint infections.

Bacteriophage-derived lysins offer a novel therapeutic approach using bacterial species-specific enzymes that hydrolyze the peptidoglycan of the bacterial cell wall (6, 7). As direct lytic agents, lysins do not require bacteria to be actively growing to exert their activity and act immediately upon contact with bacterial cells, thus rendering them as promising potential therapeutics for bone and joint infections (8). Exebacase (CF-301) is a recombinantly produced lytic enzyme derived within a *Streptococcus suis* prophage (6, 7), with a catalytic N-terminal domain linked to a cell wall-binding C-terminal domain with D-alanyl-L-glycyl endopeptidase activity (6, 7). Exebacase may have antibiofilm activity as a result of its causing bacteriolysis within biofilms as a result of its cleavage of peptidoglycan in the structural framework (8). *In vitro*, exebacase is rapidly bactericidal, shows minimal resistance development, and has synergistic activity

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with daptomycin and vancomycin against *S. aureus* (6, 8). In an experimental murine *S. aureus* bacteremia model, a single intravenous dose of exebacase with and without vancomycin or daptomycin increased survival (6). The results of a phase 2 clinical trial demonstrate a 43% higher clinical response rate with a single dose of exebacase used in addition to standard-of-care antimicrobials versus antimicrobials alone for MRSA bacteremia, including endocarditis (9, 10). We hypothesized that exebacase would be active against MRSA in experimental acute osteomyelitis in rats.

The study strain, MRSA IDRL-6169, had MICs of 0.5 μg/ml for both exebacase and daptomycin, as determined by broth microdilution (11, 12). The minimum biofilm inhibitory concentrations and minimum biofilm bactericidal concentrations were 1 and 4 μg/ml for exebacase and 1 and 2 μg/ml for daptomycin, as determined using previously described methods (14). Exebacase testing was supplemented with 0.5 mM DL-dithiothreitol and 25% horse serum (6).

The studies described were approved by the Institutional Animal Care and Use Committee of the Mayo Clinic. Osteomyelitis was established in 64 male Sprague Dawley rats using a modification of Zak's model of experimental osteomyelitis designed to mimic human infection (15). Animals were anesthetized with isoflurane and the left knee shaved and disinfected with chlorohexidine. To induce osteomyelitis, the knee joint was bent at a 45° angle to expose the top of the tibial process. A 1-ml syringe with a 21 G needle containing 10 μl arachidonic acid (50 μg/ml) and 50 μl of a 10^7 CFU suspension of IDRL-6169 in tryptic soy broth was inserted into the tibia. The bacterial suspension was slowly injected into the tibia, the needle removed, the knee joint straightened, and pressure placed on the injection site for 1 min. One week after establishing infection (day 8), rats were randomly assigned to one of four treatment arms, as follows: (i) no treatment, (ii) 60 mg/kg of body weight daptomycin intraperitoneally every 12 h (16) for 4 days, (iii) single-dose 40 mg/kg exebacase in the tail vein, or (iv) single-dose 40 mg/kg exebacase plus 60 mg/kg daptomycin intraperitoneally every 12 h for 4 days. Daptomycin was administered 15 min prior to exebacase injection. Exebacase, formulated for clinical use, was maintained on ice until injection. Rats were sacrificed 4 days after starting therapy (day 12). The left tibia from each animal was collected, weighed, and cryopulverized for quantitative bacterial culture (15). The results of quantitative cultures were compared using SAS software version 9.4 (SAS, Inc., Cary, NC) using the Kruskal-Wallis test as well as Bonferroni correction for multiple comparisons. Means and standard deviations (SD) were reported as $\log_{10}$ CFU/gram of bone. All tests were two sided; *P* values less than 0.05 were considered statistically significant.

Rats receiving no treatment had a mean ($\pm$SD) bacterial density of 5.13 ($\pm$0.34) $\log_{10}$ CFU/gram of bone. Rats in the daptomycin, exebacase, and daptomycin plus exebacase groups had mean ($\pm$SD) bacterial densities of 4.09 ($\pm$0.37), 4.65 ($\pm$0.65), and 3.57 ($\pm$0.48) $\log_{10}$ CFU/gram of bone, respectively (Fig. 1). The colony counts in all treatment groups were lower than those of the untreated rats ($P \leq 0.0001$). Daptomycin combined with exebacase-treated animals had lower colony counts than did those treated with daptomycin ($P = 0.0042$) or exebacase ($P < 0.0001$) alone. The conclusions from the study remain unchanged after application of the Bonferroni correction for multiple comparisons.

The animal model used here is one of acute MRSA osteomyelitis. *S. aureus* bone and joint infections are challenging to treat, partly due to the ability of *S. aureus* to survive in bone tissue (2). Antimicrobials such as vancomycin may not penetrate bone tissue well (17, 18), yet they are often used to treat osteomyelitis (4). It has been suggested that daptomycin has bone penetration (19). We found levels of exebacase in bone to be ~15% of plasma levels after a single dose of 10 mg/kg. As determined by enzyme-linked immunosorbent assay (ELISA), the maximum concentration in serum (C_{max}) and area under the concentration-time curve (AUC) in plasma and bone were 88 μg/ml and 12 μg/g and 28 μg·h/ml and 4 μg·h/g, respectively. Exebacase may therefore offer a strategy to target *S. aureus* bone and joint infections through its ability to lyse *S. aureus* at the site of infection. It was previously shown that exebacase is synergistic with

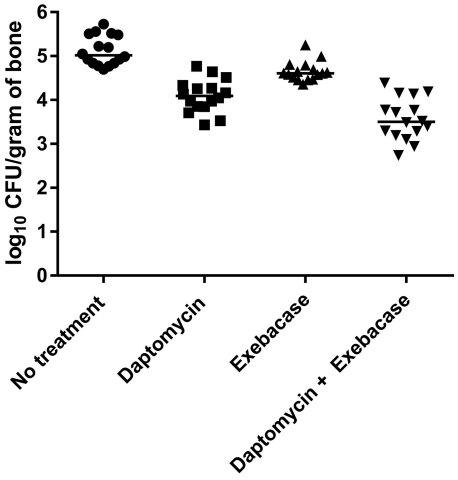

FIG 1 Results of quantitative cultures of the tibias after treatment ($\log_{10}$ CFU of MRSA/gram of bone). All treatment groups had lower bacterial loads than those with no treatment ($P \leq 0.0001$), with exebacase combined with daptomycin resulting in lower bacterial loads than those with monotherapy with either exebacase ($P < 0.0001$) or daptomycin ($P = 0.0042$). The horizontal lines depict median values.

daptomycin, possibly by increasing the ability of daptomycin to bind to its target (6). Thus, the use of exebacase plus daptomycin may offer a treatment option for acute osteomyelitis.

In this study, while treatment with daptomycin or exebacase alone showed a reduction in bacterial counts, exebacase in addition to daptomycin showed a more pronounced effect.

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