Diminished Macrophage Inflammatory Response to Staphylococcus aureus Isolates Exposed to Daptomycin versus Vancomycin or Oxacillin

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Exposure of any of six clinical isolates of Staphylococcus aureus to daptomycin alone or in combination with vancomycin or oxacillin (compared with vancomycin or oxacillin alone) led to a dampened macrophage inflammatory response with diminished tumor necrosis factor secretion and reduced accumulation of inducible nitric oxide synthase protein.

Staphylococcal infections are often associated with a severe and prolonged inflammatory response, and this has been particularly evident in children with serious infections caused by community-acquired strains of methicillin-resistant Staphylococcus aureus (CA-MRSA) (2, 4, 6, 8–11). Exposure of macrophages to bacterial products triggers the production of key inflammatory mediators including tumor necrosis factor (TNF) and, via induction of the inducible nitric oxide synthase (iNOS) protein, nitric oxide. Excessive production of these inflammatory mediators may lead to tissue injury during sepsis and meningitis.

Daptomycin is a novel cyclic lipopeptide antimicrobial active against resistant gram-positive pathogens including MRSA and vancomycin-resistant enterococci (5, 15). It has a novel method of action (1) and exhibits rapid concentration-dependent bactericidal activity without apparent bacterial lysis (7, 15). We hypothesized that exposure of staphylococci to daptomycin would result in reduced production of TNF and iNOS by macrophages compared with exposure to either vancomycin or oxacillin.

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Six staphylococcal isolates were obtained from the microbiology laboratory at Le Bonheur Children’s Medical Center (LBCMC) (4). Isolates C1 and C2 represented CA-MRSA (USA 300), isolates H1 and H2 represented hospital-acquired MRSA (HA-MRSA) (USA 100, USA 200), and isolates S1 and S2 represented methicillin-susceptible Staphylococcus aureus (MSSA). Bacteria were grown at 37°C in tryptc broth (Becton Dickinson and Co., Sparks, MD) and washed three times in endotoxin-free phosphate-buffered saline, and concentrations were determined by colony counts.

RAW 264.7 cells were purchased from the ATCC and cultured in Dulbecco’s modified Eagle’s medium (Mediatech Inc., Herndon, VA) supplemented with 10% fetal bovine serum and 2 mM l-glutamine. Experiments were done in 6-well tissue culture plates (Becton Dickinson, Lincoln Park, NJ) with 4 × 10^6 to 4.7 × 10^6 cells/well or in 24-well tissue culture plates with 1 × 10^6 cells/well. An antibiotic(s) was added to the cell cultures immediately before the addition of live staphylococci (10^6 to 10^7 CFU/ml); then cells were incubated for 18 h. In studies of iNOS protein accumulation, low concentrations of recombinant gamma interferon (rIFN-γ) (Sigma, St. Louis, MO) were added (3, 14).

Daptomycin was obtained from Cubist Pharmaceuticals (Lexington, MA). Vancomycin and oxacillin for injection were purchased from the Department of Pharmacy at LBCMC. Clinically achievable peak concentrations of antibiotics were used: daptomycin at 20 μg/ml, vancomycin at 20 μg/ml, and oxacillin at 40 μg/ml. MICs were determined by the microbiology laboratory at LBCMC using broth microdilution or Kirby-Bauer disk susceptibility testing, as recommended (12). All isolates were susceptible to vancomycin and daptomycin.

After incubation, cell supernatants were collected and assayed for TNF concentrations by using a solid-phase sandwich enzyme-linked immunosorbent assay as specified by the manufacturer (R & D Systems, Minneapolis, MN). Unpaired two-tailed t tests were used to compare TNF secretion by cells stimulated with each bacterial isolate in the presence of different antibiotics (a P value of <0.05 was considered significant). Cell lysates were prepared as described previously (3, 14) and were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to nitrocellulose membranes, and reacted with a murine monoclonal antibody specific for iNOS (Transduction Laboratories, Lexington, KY) followed by a sheep anti-mouse immunoglobulin G peroxidase-linked conjugate (Amersham, Airlington Heights, IL). iNOS protein was detected by enhanced chemiluminescence (Amersham), and band intensities were quantitated using a Bio-Rad model GS-700 densitometer.

None of the tested concentrations of daptomycin, oxacillin, or vancomycin (alone or in combination) affected TNF secretion or iNOS protein accumulation in RAW 264.7 cells stimulated with lipopolysaccharide or CpG DNA with or without rIFN-γ (data not shown) (n = 3).

Exposure of RAW 264.7 cells to each of the six Staphylococcus aureus isolates in the presence of daptomycin resulted in substantially less TNF secretion than exposure to vancomycin (all strains) or oxacillin (MSSA strains) (Fig. 1).
For each of the six staphylococcal isolates, exposure to daptomycin resulted in significantly less macrophage TNF secretion: 43% and 59% less than vancomycin for isolates C1 and C2 (n = 10056) (P < 0.05) (Fig. 1A), 45% and 44% less than vancomycin for isolates H1 and H2 (n = 4 to 5) (P < 0.05) (Fig. 1B), and 44% and 38% less than vancomycin and 51% and 41% less than oxacillin for isolates S1 and S2 (n = 4) (P < 0.05) (Fig. 1C).

Similarly, exposure of RAW 264.7 cells to each of the six staphylococcal isolates in the presence of daptomycin resulted in substantially less iNOS protein accumulation than exposure to vancomycin (MRSA strains) or oxacillin (MSSA strains) (Fig. 2). By densitometry, iNOS protein accumulation in RAW 264.7 cells stimulated with daptomycin-exposed staphylococci was reduced by 59% (compared with vancomycin [n = 25; P < 0.05]) to 74% (compared with oxacillin [n = 6; P < 0.05]).

Finally, the addition of daptomycin to vancomycin or oxacillin also resulted in comparable reductions in macrophage TNF secretion and iNOS protein accumulation in response to each of the six S. aureus isolates (Fig. 3). Addition of daptomycin to vancomycin led to reductions in TNF secretion of 64% (C1, C2), 60% (H1, H2), and 37% (S1, S2), while addition of daptomycin to oxacillin resulted in a 36% reduction in TNF secretion (P < 0.05 for each comparison). Finally, the addition of daptomycin to vancomycin or oxacillin resulted in diminished accumulation of iNOS protein in macrophages stimulated with any of the six S. aureus isolates (Fig. 3D shows representative data for isolates C1, H1, and S1). By densitometry, the average magnitude of the reduction in iNOS protein accumulation (64% [n = 13; P < 0.05]) was comparable to that observed in the presence of daptomycin alone (59 to 74%).

Stimulation of RAW 264.7 murine macrophages with each of six clinical staphylococcal isolates exposed to daptomycin singly or in combination with vancomycin or oxacillin resulted in substantially less TNF secretion and iNOS protein accumulation than was observed with exposure to vancomycin or oxacillin alone. The mechanisms responsible for this effect of daptomycin are not known but may include the diminished release of proinflammatory bacterial components (13). Our data suggest that treatment of staphylococcal infections with daptomycin (alone or in combination with vancomycin or oxacillin) might lead to a dampened host inflammatory response. The potential impact of this effect deserves additional study in animal models and in clinical trials of daptomycin therapy for staphylococcal infections.
FIG. 3. Addition of daptomycin (DAP) to vancomycin (VAN) or oxacillin (OXA) resulted in reduced TNF secretion and iNOS protein accumulation by RAW 264.7 cells stimulated with S. aureus isolates. CA-MRSA, HA-MRSA, or MSSA isolates were added to RAW 264.7 cells at final concentrations of $10^7$ CFU/ml in the presence of either vancomycin at 20 $\mu$g/ml, oxacillin at 40 $\mu$g/ml, daptomycin at 20 $\mu$g/ml, or a combination of daptomycin plus vancomycin (D + V) or daptomycin plus oxacillin (D + O), as indicated. For the iNOS studies, cells were incubated with 10 to 25 U of rIFN-γ/ml. After 18 h of incubation, supernatants were collected, and lysates were prepared and analyzed for TNF and iNOS contents, respectively. (A to C) TNF results are depicted in picograms per milliliter as means ± standard deviations. *, $P < 0.05$. Combined data for strains C1 and C2 (A), H1 and H2 (B), and S1 and S2 (C) are shown. (D) Representative iNOS immunoblots for strains C1, H1, and S1.

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