Activity of Daptomycin with or without 25 Percent Ethanol Compared to Combinations of Minocycline, EDTA, and 25 Percent Ethanol against Methicillin-Resistant *Staphylococcus aureus* Isolates Embedded in Biofilm

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Central venous catheters commonly develop central line-associated bloodstream infections. *In vitro* antibiotic lock therapy (ALT) was simulated on 10 methicillin-resistant *Staphylococcus aureus* (MRSA) clinical isolates imbedded in biofilm-coated silicon disks. Five days of 4-h daily exposures to daptomycin (2.5 mg/ml) in 25% ethanol or minocycline (3 mg/ml) plus 25% ethanol and 30 mg/ml EDTA resulted in significantly greater elimination of MRSA colonization than treatment with minocycline alone.

Of the 5 million central venous catheters (CVCs) inserted annually, an estimated 80,000 are associated with central line-associated bloodstream infections (CLABSIs) (1). CLABSIs are an independent risk factor for increased hospital costs and length of stay. There are four recognized routes for contamination of CVCs: (i) migration of organisms colonizing the skin at the insertion site into the catheter tract, along the catheter surface, and consequently colonization of the catheter tip (2–4); (ii) direct contamination of the catheter/catheter hub through contact with hands, contaminated fluids, or contaminated devices (5, 6); (iii) hematogenous seeding from another focus of infection (7); and (iv) rarely, contamination of intravenous infusate solutions (8). The microorganisms most commonly associated with CVC infection are *Staphylococcus aureus* (including methicillin-resistant *S. aureus* [MRSA]) and coagulase-negative staphylococci (CoNS) (9–11). Within 24 h of CVC insertion, host proteins coat the catheter, forming a conditioning film in which microorganisms may bind, forming three-dimensional extracellular structures colonizing the lumen of the CVC. Organisms embedded in this biofilm are usually refractory to treatment with systemic antibiotic therapy alone (6, 12). These organisms have reduced susceptibility to antimicrobial agents in the range of 10- to 1,000-fold compared to planktonic cells (13).

Published CLASBI guidelines recommend that infected long-term CVCs be removed if associated with severe sepsis, suppurative thrombophlebitis, endocarditis, bloodstream infection that continues despite 72 h of antimicrobial therapy to which the infecting microbes are susceptible, or infections due to *S. aureus* and that short-term CVCs be removed from patients with CLABSIs due to *S. aureus* (14). Unfortunately, on occasion, clinical circumstances may preclude removing the CVC for reasons of safety or feasibility. Antibiotic lock therapy (ALT) involves instilling a highly concentrated antibiotic solution into a catheter lumen and allowing the solution to dwell for a specified time period for the purpose of sterilizing the lumen and salvaging the CVC. In conjunction with systemic antimicrobial agents, ALT is indicated for CLABSIs involving long-term catheters with no signs of exit site or tunnel infection with the goal of catheter salvage.

Daptomycin, a novel lipopeptide antibiotic, has potent bactericidal activity *in vitro* against most clinically important Gram-

FIG 1 Colony counts after 5 days of treatment grouped by antibiotic treatment. Ten MRSA clinical isolates were treated with 4-h daily exposures to Dap at 2.5 mg/ml (Dap 2.5), Dap 2.5 + ETOH, Min at 3.0 mg/ml (Min 3.0), and Min 3.0 + ETOH/E for five consecutive days. The initial inoculum of bacteria was 5×10^5 per well. The mean values plus standard errors of the means (error bars) are shown.

positive pathogens, including MRSA, CoNS, vancomycin-resistant enterococci (VRE), and penicillin-resistant *Streptococcus pneumoniae* (PRSP) (15–19). Because daptomycin is bactericidal against the most common organisms associated with CLABSIs, including those in stationary growth phase, it may show promise as an agent for ALT (20).

The purpose of this study was to compare the activity of daptomycin with or without 25% ethanol to minocycline (3 mg/ml) with or without 25% ethanol and/or EDTA (30 mg/ml) in eradicating biofilm-producing strains of MRSA in well-studied *in vitro* models of catheter-related infection (21).

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FIG 2 Colony counts after 5 days of treatment grouped by control treatment. Ten MRSA clinical isolates were treated with 4-h daily exposures to MHB alone, ETOH, EDTA, and ETOH/E for five consecutive days. The initial inoculum of bacteria was 5×10^5 per well. The mean values plus standard errors of the means (error bars) are shown.

Sterile silicone sheets (Cardiovascular Instrument Corp., Wakefield, MA) were washed with hand soap and rinsed with distilled water. Disks with a diameter of 1.5 cm were cut from the silicon sheets with a cork borer, autoclaved, and aseptically transferred to sterile 12-well tissue culture plates (Becton, Dickinson, Franklin Lakes, NJ) containing 1 ml human plasma and incubated with shaking for 24 h at 37°C (13, 15). Plasma was then replaced with a 1-ml bacterial inoculum from 1 of 10 clinical MRSA isolates adjusted to 5.5×10^5 organisms/ml, using McFarland turbidity standards, in Muller-Hinton broth (MHB), and incubated with shaking for 24 h at 37°C. The inoculated broth was removed, and the disks were washed once with 0.9% saline and shaking for 30 min. The disks were then transferred into new tubes containing either MHB alone or MHB containing one of the following: (i) daptomycin at a concentration of 2.5 mg/ml (Dap 2.5), (ii) daptomycin at 2.5 mg/ml in 25% ethanol (Dap 2.5+ETOH), (iii) minocycline at 3 mg/ml (Min), (iv) minocycline at a concentration of 3 mg/ml in 25% ethanol (Min+ETOH), or (v) minocycline at 3 mg/ml in 25% ethanol and EDTA (30 mg/ml) (Min+ETOH/E) for 4 h. All daptomycin was supplemented to a physiologic level of 50 mg/liter Ca^{2+} . At the end of 4 h (a time interval chosen to mimic a feasible clinical interval), two disks from each group were placed in 5 ml of 0.9% saline, sonicated for 15 min, and vortexed for 30 s, and a 50- μ l aliquot was plated onto Trypticase soy agar with 5% sheep blood using a spiral plating device (spiral plater model D; Advanced Instruments, Norwood, MA). The plates were incubated overnight at 37°C. Colonies were counted with an automatic colony counter (aCOLyte colony counter; Synbiosis USA, Frederick, MD), and final counts were calculated with dilution factors taken into account. All plates with no growth were held for an additional 24 h. The remaining silicone disks were reincubated for 24 h in MHB alone before they were reexposed to antibiotic. This protocol was repeated for a total of 5 consecutive days with two disks removed daily for culturing.

Clinical isolates treated for 5 days with Dap 2.5, Dap 2.5+ETOH, or Min+ETOH/E showed a statistically significant reduction of colony counts compared to treatment with Min alone ($P = 0.039$) (Fig. 1; controls shown in Fig. 2.) All computations were performed using the StatSoft Statistica program (StatSoft, Inc., Tulsa, OK). Activity of the drug combinations mentioned above (indicated by mean CFU) were compared using a one-way analysis of variance (ANOVA) test and a *post hoc* Fisher

least significance difference (LSD) test ($P < 0.05$ was considered statistically significant).

The results of this preliminary study show that MRSA clinical isolates incubated for 4 h daily with daptomycin (2.5 mg/ml) with and without 25% ethanol or with minocycline plus ETOH/E resulted in significant reduction of MRSA colony counts compared to those incubated in minocycline (3 mg/ml) alone after 5 days of exposure. Future investigation might include testing higher concentrations of daptomycin solutions with and without 25% ethanol in this model to identify the most potent concentration against *S. aureus* followed by controlled clinical trials evaluating the use of daptomycin to salvage CVCs with CLABSIs.

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