Rifampicin enhances activity of daptomycin and vancomycin against both a polysaccharide intercellular adhesin (PIA)-dependent and -independent Staphylococcus epidermidis biofilm

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Objectives and methods: This study addressed the efficacy of daptomycin, vancomycin, rifampicin, daptomycin/rifampicin and vancomycin/rifampicin against a polysaccharide intercellular adhesin (PIA)-dependent and -independent Staphylococcus epidermidis biofilm using flow cell and guinea pig tissue cage models.

Results: The flow cell model of both PIA-dependent and -independent biofilms demonstrated that the viable cell count after treatment with daptomycin/rifampicin was significantly lower (P<0.05) than after treatment with vancomycin, vancomycin/rifampicin, daptomycin or rifampicin alone. To validate these observations, a guinea pig tissue cage model was used. The results demonstrated that the addition of rifampicin to daptomycin or vancomycin sterilized 5/6 tissues cages colonized with S. epidermidis 1457 (PIA producing). Similar results were noted with S. epidermidis 1457 icaADBC::dhfr (non-PIA producing), where daptomycin/rifampicin and vancomycin/rifampicin sterilized 5/6 and 6/6 tissue cages, respectively. There was no statistical difference in comparison with the no-treatment control when both 1457 and 1457 icaADBC::dhfr were treated with vancomycin and daptomycin alone. Furthermore, treatment with rifampicin alone sterilized 5/6 and 3/6 1457 and 1457 icaADBC::dhfr tissue cages, respectively.

Conclusions: Interpretation of these data suggests that rifampicin is highly active against S. epidermidis biofilms and both vancomycin and daptomycin are effective at reducing the subpopulation of bacteria that develop rifampicin resistance.

Keywords: biomaterial infections, guinea pig model, tissue cage, icaADBC operon

Introduction

Staphylococcus epidermidis is the most prevalent cause of hospital-acquired infections.1 A majority of S. epidermidis infections are associated with biomaterials. Adherence to these biomaterials and subsequent biofilm synthesis are defined virulence factors as assessed by relevant animal models of infection.2–5 A major component of S. epidermidis biofilm is polysaccharide intercellular adhesin (PIA), which is synthesized by enzymes encoded by the icaADBC operon.6,7 PIA contributes to initial adherence in addition to intercellular accumulation and structural rigidity.8 Furthermore, PIA protects against major components of the human innate host immune system and was recently shown to be responsible for pro-inflammatory cytokine production.9,10

It is clear that, in contrast to Staphylococcus aureus, not all S. epidermidis isolates encode the icaADBC locus.11–15 Therefore, in these icaADBC-negative isolates, biofilm formation and intercellular accumulation are thought to be mediated by extracellular DNA16,17 and other proteinaceous factors, including accumulation-associated protein (Aap),18 biofilm-associated protein (Bhp)19 and the extracellular matrix binding protein (Ebh).20,21 Accordingly, Rohde et al.14 found that 27% of biofilm-positive S. epidermidis isolates obtained from prosthetic joint infections following total hip or knee replacement produced PIA-independent biofilms. Although it is still unclear whether the substructure metabolism is different between PIA-dependent and -independent biofilms, it is well established that bacteria growing in a biofilm state are more recalcitrant to antibiotics. Multiple oxygen and nutrient gradients have been identified within biofilms, allowing unique niche metabolism.22,23 Further complicating antibiotic treatment of biofilm-associated infections includes the development of persister cells24 and the
horizontal acquisition of antibiotic resistance determinants, which narrow the available treatment options. Approximately 85% of S. epidermidis clinical isolates are resistant to oxacillin; therefore, vancomycin and daptomycin are commonly used treatment modalities against biomaterial-related infections. This study investigated the efficacy of vancomycin and daptomycin alone and in combination with rifampicin in the treatment of an S. epidermidis biomaterial-based infection in a guinea pig tissue cage model and an in vitro flow cell biofilm model. The contribution of PIA to the recalcitrance of biofilms to antibiotics was addressed by including an isogenic PIA-negative (1457 icaΔDBC::dhfr) S. epidermidis strain in both the guinea pig and the flow cell model.

Materials and methods

S. epidermidis strains

The S. epidermidis strains used in the study were S. epidermidis 1457 and the previously constructed isogenic, PIA-negative strain 1457 icaΔDBC::dhfr.25

Microscopy

Scanning electron microscopy (EM) was performed using an FEI Quanta 200 (Hillsboro, OR, USA) at the University of Nebraska Medical Center Electron Microscopy Core Laboratory.

Flow cell biofilm model

Plastic convertible Stovall flow cells (24 mm x 40 mm x 8 mm; Stovall, Greensboro, NC, USA) were used to test an in vitro biofilm antibiotic treatment model. Six flow cells were used in each experiment: three control cells (not treated with antibiotics) and three experimental cells (treated with antibiotics). The three control and experimental flow cells were connected to separate 10 L carboys containing tryptic soy broth (TSB; Difco, Detroit, MI, USA) to ensure sterility and each tissue cage was inoculated with S. epidermidis 1457 icaΔDBC::dhfr grown in TSB to an optical density (600 nm) of 0.5 and 100 μL was subsequently added to each flow cell; the bacteria were allowed to adhere to the flow cell for 30 min before initiation of the flow (flow rate = 0.5 mL/min). The biofilms were allowed to mature for 48 h, at which time the antibiotic was added to the one carboy supplying TSB to the three experimental flow cells. The viable cell count (cfu/mL) was determined from the control flow cells at 48, 72 and 96 h timepoints by resuspension of the biofilms in 1 mL of PBS, serially diluted and plated onto tryptic soy agar plates to reveal cfu/mL (limit of detection, 10 cfu). Statistical analysis was performed using the Mann–Whitney test. P values of < 0.05 were considered statistically significant. The University of Nebraska Medical Center Institutional Animal Care and Use Committee (IACUC) approved these experiments.

Results

In vitro biofilm flow cell antibiotic treatment model

An in vitro flow cell biofilm model was developed to determine the antibiotic efficacy of daptomycin, vancomycin, rifampicin, vancomycin/rifampicin and daptomycin/rifampicin against PIA-dependent and -independent biofilms. Flow cells were allowed to mature for 48 h, at which time three of the six flow cells were treated with one of the aforementioned antibiotics or antibiotic combinations. As shown in Figure 1, untreated, PIA-dependent biofilms developed significant three-dimensional structure from 48 to 96 h of growth. 1457 icaΔDBC::dhfr grown in the flow cell setting were devoid of structure formation and appeared as a flat mat (data not shown).24-26 However, after treatment with any of the antibiotics (vancomycin, daptomycin or rifampicin) for 48 h, the visible three-dimensional structure did not fully develop (Figure 1) and the viable cell count decreased significantly (Figure 2). After 96 h of growth, the mean control viable cell count for the PIA-dependent biofilm (strain 1457) was 2 x 10^7 cfu/mL (from 3 x 10^8 cfu/mL after 48 h of growth). Treatment with daptomycin, vancomycin, vancomycin/rifampicin, daptomycin/rifampicin or rifampicin significantly (P < 0.05) decreased the viable cell count after 48 h of treatment in comparison with the 96 h control (Figure 2). However, the viable cell count after treatment with daptomycin/rifampicin was
significantly lower \((P<0.05)\) than that after treatment with vancomycin, vancomycin/rifampicin, daptomycin or rifampicin. Similar observations were noted using 1457 icaADBC::dhfr in the flow cell system. A viable cell count of \(2 \times 10^{10}\) cfu/mL was acquired after 96 h of growth; treatment with all five antibiotics/antibiotic combinations significantly decreased the viable cell count after 48 h of antibiotic treatment (Figure 3). However, as observed with 1457, the viable cell count of 1457 icaADBC::dhfr after treatment with daptomycin/rifampicin was significantly lower than after treatment with any other antibiotic.

Figure 1. Growth of \(S.\) epidermidis 1457 in a flow cell chamber. \(S.\) epidermidis 1457 biofilm at (a) 48 h and (b) 96 h of growth. Note significant three-dimensional structure of the biofilm after 96 h of growth. (c) \(S.\) epidermidis 1457 after vancomycin/rifampicin treatment for 48 h. Note lack of three-dimensional structure in comparison with (b). Arrow on right-hand side of figure denotes direction of the media flow.

Figure 2. In vitro flow cell experiments with \(S.\) epidermidis 1457. The white bar represents mean cfu/mL after 48 h of growth (time 0) before addition of antibiotic. The black bar represents the mean cfu/mL of the untreated flow cells after an additional 48 h of growth (96 h of growth). The grey bars represent the mean cfu/mL after 48 h of exposure to the indicated antibiotic. *Significance \((P<0.05)\) as compared with the 96 h timepoint. **The antibiotic treatment regimen was significantly different from all other treatments tested. VAN, vancomycin; RIF, rifampicin; DAP, daptomycin.
In fact, no colonies were detected (limit of detection, 10 cfu). This experiment was repeated twice (n=6 flow cells in total) with identical results. To determine whether rifampicin-resistant mutants were selected during rifampicin treatment, 100 colonies were isolated from both 1457 and 1457 icaADBC::dhfr flow cells treated with rifampicin and colonies were transferred to TSA plates containing 10 mg/L rifampicin. As predicted, all 100 colonies grew on TSA containing rifampicin, suggesting that the failure of rifampicin to eradicate the biofilm was due to the selection of rifampicin-resistant mutants.

Guinea pig tissue cage model
In order to extend our in vitro observations and more closely mimic the human infection, a guinea pig tissue cage model was used. Pilot studies were performed to determine the smallest inoculum of both S. epidermidis 1457 and 1457 icaADBC::dhfr that would colonize each tissue cage. It was determined that an inoculum of 5×10^5 cfu of strain 1457 was reliably able to colonize each guinea pig tissue cage. In addition, we found that the tissue cages remained colonized for at least 28 days (~5×10^6 cfu/mL of tissue cage aspirate) and the infection did not metastasize to other organ systems, including the liver, kidney, heart or spleen; therefore, this model mimics a typical indolent S. epidermidis biomaterial-related infection seen in humans. Scanning EM verified that both the tissue cages and the tissue cage glass beads were covered with biofilm following colonization with strain 1457 (Figure 4a and b). Following confirmation that the tissue cages were colonized with S. epidermidis 1457 (5 days post-inoculation), three animals each were treated with daptomycin, vancomycin, rifampicin, daptomycin/rifampicin and vancomycin/rifampicin for 5 days. Six animals (n=12 tissue cages) were used as untreated controls. Following sacrifice of the animals and obtaining the viable count from the glass beads, it was determined that there was no statistical difference between the control group and the animals that were treated with vancomycin and daptomycin alone (Figure 5). The mean cfu/mL in the control group, animals treated with daptomycin and those treated with vancomycin was 3.76×10^4, 1.16×10^4 and 7.88×10^4 cfu/mL, respectively. In contrast, no bacteria were detected following treatment with rifampicin in 5/6 tissue cages. Furthermore, treatment with daptomycin/rifampicin and
vancomycin/rifampicin also sterilized 5/6 tissue cages. To determine whether rifampicin-resistant mutants were selected during therapy with rifampicin, rifampicin/daptomycin and rifampicin/vancomycin, 100 colonies were picked from the three non-sterile tissue cages (one from each treatment arm) and transferred to TSA containing 10 mg/L rifampicin. It was found that, in all three cases, 100/100 colonies grew on media containing rifampicin, suggesting that the selection of rifampicin-resistant mutants during treatment complicated the treatment regimen.

In contrast to strain 1457, pilot studies demonstrated that a higher inoculum of 1457 icaADBC::dhfr \(5 \times 10^7\) cfu was needed to colonize 100% of the tissue cages. However, a similar level of colonization in strains 1457 and 1457 icaADBC::dhfr \(3.76 \times 10^6\) cfu/mL and \(1.45 \times 10^6\) cfu/mL, respectively) was observed in terms of cfu/mL extracted from the tissue cage beads following animal sacrifice (Figure 6). Similar to 1457, it was determined that there was no significant difference between the control group of 1457 icaADBC::dhfr and the vancomycin treatment group (mean \(1.45 \times 10^6\) and \(1.18 \times 10^6\) cfu/mL, respectively). Treatment with daptomycin alone against tissue cages colonized with 1457 icaADBC::dhfr resulted in a mean \(1.66 \times 10^3\) cfu/mL per tissue cage; however, this result did not reach statistical significance \((P=0.06)\) (Figure 6). Treatment with rifampicin, daptomycin/rifampicin and vancomycin/rifampicin sterilized 3/6, 5/6 and 6/6 tissue cages, respectively. As observed with strain 1457, all colonies tested \((n=100\) per tissue cage\) of 1457 icaADBC::dhfr recovered from the non-sterile tissue cages from all three treatment arms containing rifampicin were resistant to rifampicin.

Figure 5. Efficacy of antibiotics against S. epidermidis 1457 in a guinea pig tissue cage model. Black bars represent cfu recovered from each individual tissue cage. Grey bars represent the mean and SEM of each dataset. *Significance \((P<0.05)\) as compared with the no-treatment control.

Figure 6. Efficacy of antibiotics against S. epidermidis 1457 icaADBC::dhfr in a guinea pig tissue cage model. Black bars represent cfu recovered from each individual tissue cage. Grey bars represent the mean and SEM of each dataset. *Significance \((P<0.05)\) as compared with the no-treatment control.
Discussion

Daptomycin is a cyclic lipopeptide antibiotic that is active against Gram-positive bacteria and is especially useful against highly resistant pathogens such as methicillin-resistant S. aureus and vancomycin-resistant Enterococcus faecium. Daptomycin is a calcium-dependent antibiotic that causes membrane disruption and depolarization, potassium ion leakage and eventual cell death.28,29 However, the efficacy of daptomycin in the treatment of S. epidermidis biofilms is unclear. Although the effectiveness of daptomycin has been addressed in a catheter lock model30 and in an experimental model of endocarditis,31 this is the first study to address the effectiveness of daptomycin and rifampicin against well-defined PIA-dependent and -independent S. epidermidis biofilms in in vitro and in vivo models.

Due to the lack of randomized clinical trials, there are no standard clinical practices regarding the treatment of S. epidermidis biofilm-associated infections. Although rifampicin is never used clinically as monotherapy due to rapid development of resistance,12–34 many studies have demonstrated its effectiveness against staphylococcal biofilms in combination with other anti-staphylococcal antibiotics, including tigecycline,35,36 trimethoprim/sulfamethoxazole,36,37 linezolid,38–40 quinupristin/dalfopristin,39,41 ciprofloxacin42,43 and levofloxacin.44 For instance, one blinded, randomized clinical trial for the treatment of orthopaedic implant infections comparing ciprofloxacin against ciprofloxacin plus rifampicin demonstrated that the ciprofloxacin/rifampicin arm had a clinical cure rate of 100% versus 58% in the ciprofloxacin only arm.43

This study was designed to address the efficacy of daptomycin and vancomycin in the treatment of both PIA-dependent and -independent biofilms. Because of the aforementioned efficacy against staphylococcal biofilms, rifampicin was added to both vancomycin and daptomycin to determine whether it significantly altered the viability of bacteria growing in a biofilm. First, we found that both vancomycin and daptomycin alone were not effective in the treatment of an S. epidermidis-mediated foreign body infection (i.e. tissue cage). Similar results were noted for both 1457 and 1457 icaADBC::dhfr. Several studies have demonstrated that daptomycin, vancomycin and rifampicin rapidly penetrate staphylococcal biofilms; thus, their inability to inhibit growth is most likely due to the quiescent state of the bacteria in the biofilm.55–50 However, both antibiotics in combination with rifampicin were highly efficacious in sterilizing tissue cages colonized by both strains 1457 and 1457 icaADBC::dhfr. Surprisingly, no bacteria were detected in 5/6 and 3/6 tissue cages colonized by 1457 and 1457 icaADBC::dhfr, respectively, following treatment with rifampicin alone. However, predictably, colonies that were isolated from the non-sterile tissue cages were resistant to rifampicin, further demonstrating that rifampicin should not be used as monotherapy due to rapid development of resistance. In fact, all colonies isolated from any tissue cage treated with rifampicin (either alone or in combination with vancomycin or daptomycin) were resistant to rifampicin (data not shown). Interpretation of these data suggests that rifampicin is highly active against staphylococcal biofilms and that the utility of either vancomycin or daptomycin in combination with rifampicin is to inhibit the outgrowth of rifampicin-resistant mutants that are selected during therapy. Unfortunately, experiments were not performed to determine whether, following successful therapy, the tissue cages would again become colonized by either S. epidermidis 1457 or 1457 icaADBC::dhfr. Further experimentation will address questions regarding the function of persisters in S. epidermidis biomaterial-related infections.

The development of an in vitro flow cell model of antibiotic therapy was advantageous for two reasons. First, since the mean number of bacteria detected in the guinea pig tissue model was ∼5×10^4 cfu/mL, we wanted to create a model where the number of bacteria being treated was significantly greater. Second, using a treatment model with a higher inoculum, we hypothesized that subtle differences between the treatment efficacy of vancomycin/rifampicin and daptomycin/rifampicin would be detected. The in vitro flow cell model demonstrated that daptomycin/rifampicin was more effective in the treatment of both a PIA-dependent and -independent biofilm. Surprisingly, however, it was determined that daptomycin/rifampicin sterilized flow cells colonized with 1457 icaADBC::dhfr. These results may suggest that PIA is protective against the deleterious action of antibiotics or, alternatively, that PIA provides specific niches that allow antibiotic tolerance or persister cells to develop. In addition, it is suggested that rifampicin was not effective at clearing the flow cell biofilm due to the high bacterial burden and subsequent increased chance of selection of rifampicin-resistant mutants.

In conclusion, the in vivo guinea pig tissue cage model demonstrated that rifampicin in combination with either vancomycin or daptomycin is highly active against S. epidermidis biofilm-mediated infections; this approach deserves greater clinical scrutiny. These results suggest that RNA metabolism is important for biofilm growth and, possibly, that RNA polymerase is an effective target for antibiotic development against bacteria that commonly grow in a biofilm. Furthermore, our in vitro flow cell model demonstrated that the combination of daptomycin/rifampicin is superior to vancomycin/rifampicin against both a PIA-dependent and a PIA-independent biofilm. Lastly, our in vitro results suggest that PIA protects against the combination of daptomycin and rifampicin, suggesting a function that counteracts, in an unknown manner, the deleterious action of antibiotics against the bacterium.

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Transparency declarations

M. E. R. serves on a Cubist Pharmaceuticals advisory board. All other authors: none to declare.

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