ABSTRACT

Medical device-associated infections represent a growing problem with limited or no therapeutic options beyond implant removal. Bacterial biofilm is the major and the final determinant of the poor prognosis of these difficult-to-treat infections. Due to the high antimicrobial resistance level of bacteria organized in biofilms, combination therapy is most often recommended, and macrolides may represent antibiotics of choice. Their anti-biofilm activity has been successfully used in-vitro and in-vivo against biofilm-associated infections caused by Pseudomonas aeruginosa and other Gram-negative bacilli. However there is little data regarding their clinical interest against infections involving staphylococcal biofilms. Despite controversial reports, there is growing in-vitro and in-vivo evidences of anti-staphylococcal biofilm activity of macrolides that could represent a significant advance in the battle against implant-related infections.

INTRODUCTION

Management of medical device associated infections, such as prosthetic heart valves, cardiac pacemakers, and prosthetic joint replacements, has become a conventional procedure of modern medical care. The use of foreign material however, is not free of complications with bacterial infections being the most devastating adverse event and Staphylococci spp. isolates being the most common pathogens involved (table 1). Biofilm producing bacteria are capable of adhesion to foreign body materials, forming organized community of adherent cells living in layers of slime. Once mature, the bacterial biofilm developed on implanted medical devices exhibits an increased tolerance to antimicrobial agents, increased withstanding of physical forces (including shear forces produced by blood or surgical lavage) and an extraordinary resistance to phagocytosis. Therapeutic options available to treat biofilm-associated infections are therefore limited, in most cases, to the removal of the infected device. Despite the advances in understanding the properties of biofilms and the development of novel technologies, prevalence, mortality and morbidity rates as well as estimated cost of biofilm associated infections have increased. Recent estimates suggest that cost of prosthetic joint infections could be up to $50,000 per patient and $250,000 million per year. This highlights the urgent need for novel therapeutic strategies to treat bacterial and yeast biofilm related infections. For instance, in prosthetic joint infections (PJI), especially late-onset PJs, combination therapy with rifampin is typically used after debridement and retention of the infected implant. Rifampin based combination has already demonstrated anti-biofilm efficacy in-vitro and in-vivo, however PJI often involved the formation of biofilm that precludes therapeutic success without surgery. Indeed,
success rates reported for implant removal for rifampin based combination therapy range between 7-80%13,14. There is an urgent need for new strategies to manage biofilm related infection; therefore, the usefulness of other anti-biofilm antimicrobials like macrolides should be investigated. Some agents of this class (erythromycin, clarithromycin and azithromycin) have already demonstrated a potent in-vitro antimicrobial properties of biofilms24. They usually come from lysis of embedded cells, but excretion of DNA cannot be excluded24. Exopolysaccharides are also essential components that intervene on the formation and maturation of bacterial biofilms24. These molecules differ from species to species and also differ from strain to strain within the same species. For example, P. aeruginosaa biofilms contain at least three major polysaccharides (alginites, Pel, Pst)25 whereas S. epidermidis and S. thermodilus produce polysaccharide intercellular adhesion (PIA), and hetero-polysaccharides of different monomer compositions, ratios and molecular weight, respectively24. In addition, a large variety of extracellular enzymes capable of cleavage of proteins, lipids and polysaccharides are present within the matrix, and play key roles in the process of formation / maturation of the matrix as well as in the virulence properties of biofilms24, 25. In general, the formation of a scaffold of biofilm matrix molecules involves five distinct steps that include initial attachment, cell-to-cell adhesion and proliferation, maturation, and finally, detachment26. Bacterial adhesion to a surface is crucial in the process of biofilm formation and involves several molecules, such as polysaccharides, proteins, eDNA and amphiphilic molecules, whereas cohesion of cells within the matrix is ensured by neutral and charged polysaccharides and proteins (such as amyloids and lectins)24,25. Molecules of eDNA enclosed within the matrix are a major structural component of P. aeruginosaa and S. aureus biofilms24,26. These molecules seem to be involved in the cohesion of embedded cells, play a role in inhibition or promotion of biofilm, and are responsible for antimicrobial properties of biofilms24. They usually come from lysis of embedded cells, but excretion of DNA cannot be excluded24. Exopolysaccharides are also essential components that intervene on the formation and maturation of bacterial biofilms24. These molecules differ from species to species and also differ from strain to strain within the same species. For examples, P. aeruginosaa biofilms contain at least three major polysaccharides (alginites, Pel, Pst)25 whereas S. epidermidis and S. thermodilus produce polysaccharide intercellular adhesion (PIA), and hetero-polysaccharides of different monomer compositions, ratios and molecular weight, respectively24. In addition, a large variety of extracellular enzymes capable of cleavage of proteins, lipids and polysaccharides are present within the matrix, and play key roles in the process of formation / maturation of the matrix as well as in the virulence properties of biofilms24, 25. In general, the formation of a scaffold of biofilm matrix molecules involves five distinct steps that include initial attachment, cell-to-cell adhesion and proliferation, maturation, and finally, detachment26. Bacterial adhesion to a surface is crucial in the process of biofilm formation and involves several molecules, such as polysaccharides, proteins, eDNA24,25. Once successfully attached, the bacterial community
confirmed these preliminary results against biofilm embedded cells of a clinical and non mucoid strain of *P. aeruginosa*. In presence of clarithromycin (concentration up to 20 mg/L), the authors observed a net reduction of the amount of alginate and hexose, both in the colonies and the environment, suggesting destruction of the polysaccharide glycocalyx and/or the inhibition of polysaccharide synthesis by clarithromycin. Despite these evidences about the anti-biofilm activity against gram-negative organisms, there is contradictory information regarding the effect of macrolides in biofilm formation of gram-positive organism. Rachid et al. reported an increased expression of the intercellular adhesion gene cluster (*ica*) which comprises 4 genes (*icaADBC*), in presence of erythromycin. Of note, a similar activity was observed for others antibiotics, such as tetracycline or the streptogramin antibiotic, quinupristin-dalfopristin, although oxacillin, penicillin G, clindamycin or gentamycin did not seem to impact biofilm formation. More recently, Wan et al. evaluated the role of macrolides in biofilm formation and its relation to the *ica* status of *S. epidermidis*. Exposure of erythromycin resistant strains of *S. epidermidis* to macrolides (at ¼ x MIC) resulted in an increase production of biofilm for 24 (20%) organisms, independently of the *ica* status, suggesting that other regulatory systems are involved in the mechanism of macrolide-enhanced biofilm formation.

**Other authors have reported that macrolides also inhibit biofilm formation of gram-positive organisms, being clarithromycin the most extensively studied macrolide against mature biofilm of *Staphylococcus* spp. One of the first experiments on this topic was performed by Yasuda et al. in 1994."
of *S. epidermidis* in a glass chamber with a cellulose membrane filter with different concentrations of clarithromycin and ofloxacin or cefotiam. They observed a clarithromycin dose-dependent (1 to 20 mg/L) increase in the rate of penetration of ofloxacin and cefotiam through the filters, suggesting the eradication of the glycocalyx matrix of biofilms by clarithromycin. Few years later, Sano et al. explored the efficacy of clarithromycin and vancomycin against *S. aureus* isolates in an in vitro model of urinary tract infection. Vancomycin and clarithromycin MIC values were 0.5 (susceptible) and > 128 mg/L (resistant), respectively. Simulated dosing regimens mimicked human pharmacokinetics of each agent. After 48 h of therapy, vancomycin eradicated methicillin-resistant *S. aureus* (MRSA) biofilm formed on the bladder model. However, withdrawal of therapy and replacement of media resulted in bacterial re-growth. As expected, clarithromycin alone had no antibacterial activity on the MRSA count. However, combination therapy resulted in a complete eradication of the MRSA within 46 h. No re-growth was observed after changing the media, suggesting eradication of persistent cells embedded in the matrix of biofilm grown on the bladder. Scanning electron microscopy confirmed persistence of biofilm with clarithromycin alone and complete eradication of biofilm and planktonic cells with combination therapy.

Similarly, Fujimura et al. reported the efficacy of clarithromycin on combination with ceftazidime or vancomycin in an in vitro model of foreign-body infection and in an in vivo model of implant-related infection. Mature biofilm of *S. aureus* biofilm were grown at the surface of titanium washers prior exposure to clarithromycin plus ceftazidime or vancomycin for 72 h. Elimination of the biofilm and complete sterilization of the media secondary to combination therapies with clarithromycin was confirmed using scanning electron microscopy, whereas monotherapy did not eradicate bacterial biofilms and did not prevent re-growth at 72 h.

More recently, we developed an *in vitro* pharmacokinetic/pharmacodynamic model of bacterial biofilm-related infections. We evaluated the activity of daptomycin and moxifloxacin alone and in combination with clarithromycin against a methicillin-susceptible, clarithromycin-resistant *S. aureus* strain. Neither daptomycin nor moxifloxacin were able to eradicate mature biofilm, but combination of any of these antimicrobial agents with clarithromycin significantly improved the antibacterial activity, with a substantial decrease of viable bacteria. Daptomycin plus clarithromycin was the most effective combination, decreasing viable bacteria below the limit of detection (10 cells/mL). Scanning electron microscopy confirmed these results, illustrating the biofilm destruction and/or eradication in presence of the combo daptomycin plus clarithromycin.

Azithromycin has also been evaluated against mature staphylococcal biofilms. For example, Presterl et al. evaluated the activity of azithromycin combined to daptomycin, vancomycin, tigecycline, fosfomycin (5 and 20 x MIC) or ceftiraxone (100 x MIC) on mature biofilms of 12 non-related strains of *S. epidermidis* under static conditions of exposure. None of the combinations were able to reduce the viable count of bacteria, although a slight decrease was observed with vancomycin and fosfomycin. Although there is limited data on azithromycin, the absence of anti-biofilm activity of this macrolide against Staphylococci suggests that this may be a specific property of clarithromycin. A recent study investigated the rate of penetration of different antimicrobial agents, including azithromycin and erythromycin, in agar layers containing 1% of *P. aeruginosa* alginate revealed that the two macrolide antibiotics has the best rate of penetration (100%), followed by ceftazidime and ciprofloxacin (90 to 95%). Further studies are warranted to explore antimicrobial

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**Table 2** *In vitro* and *in vivo* reports on macrolide combinations against Gram positive biofilm.

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penetration rate, inhibition at the surface of the biofilm matrix and how macrolide antibiotics could impact on these properties depending on the species.

**Therapeutic potential of macrolides in the treatment of biofilm related infections**

Since clinicians became aware of the anti-biofilm properties of macrolides against *Pseudomonas* biofilms, there has been a widespread use of azithromycin and clarithromycin in biofilm-associated infections caused by *Pseudomonas* spp. like cystic fibrosis or diffuse panbronquilitis. Randomized controlled trials have demonstrated respiratory benefits of long-term therapy with macrolides. The beneficial effect of macrolide therapy was evident only in patients infected with *P. aeruginosa* suggesting that the beneficial effect was not related to the immunomodulatory properties of macrolides. The same conclusion was reported very recently in a meta-analysis of randomized controlled trials of azithromycin (7 trials) and clarithromycin (1 trial) for the treatment of cystic fibrosis, where macrolide therapy was associated with improvement of lung function, especially for *P. aeruginosa*-colonized cystic fibrosis patients.

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**Figure 2** Scan Electron Microscopy imaging of the surface of the coupons to assess presence and structure of the matrix of SH1000 biofilm. Images were collected at 2,000 x magnification. A. Before any drug exposure, B. After 72 h of daptomycin exposure, C. After 72 h of daptomycin + clarithromycin exposure, D. After 72 h of moxifloxacin + clarithromycin exposure. Reproduced with permission.
However, despite that much evidence regarding macrolide therapy for biofilm-associated infections of *P. aeruginosa*, there is no data about macrolide therapy for biofilms-associated infections of *Staphylococci* and therefore no consistent recommendations can be made regarding its therapeutic potential in the treatment of this type of infections. However, because of the potential benefit, and safety of macrolides, as shown in randomized controlled trials of long term macrolide therapy10,12, clinicians should consider the addition to macrolides when treating medical-device associated infections.

CONCLUSIONS

Because of their well-described ability to disrupt alginate-based biofilms, macrolides have been extensively studied against *Pseudomonas*. In contrast, little is known regarding their interaction with staphylococcal biofilms and potential clinical interest in the treatment of *Staphylococci* infection associated with biofilms. There is growing evidences of the anti-biofilm activity of macrolides, especially clarithromycin against mature staphylococcal biofilms. Although the mechanism is not completely elucidated, inhibition of hexose-production is suggested in an MIC independent manner, similarly to what has already been described in gram-negative rods. Further investigations, especially regarding the genetic pathways (*agr, SigB, SarA, ica*, etc...) involved in the biofilm production and how antimicrobial therapy is affecting these genetic components are warranted. As *Staphylococci* are responsible of most of the medical devices-associated infections, clinical application of the anti-biofilm effect of macrolides could be of paramount importance in the treatment of those difficult-to-treat infections.

REFERENCES