Colicin-like bacteriocins as novel therapeutic agents for the treatment of chronic biofilm-mediated infection

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Abstract

The emergence of pan-resistant strains of Gram-negative pathogens and the ability of many bacteria to form multidrug-resistant biofilms during chronic infection poses the grave threat of bacterial infections that are truly untreatable with our current armoury of antibiotics. Despite obvious clinical need, few new antibiotics have entered clinical practice in recent years. For ‘difficult to treat’ Gram-negative bacteria such as Pseudomonas aeruginosa and Escherichia coli, where the presence of outer membrane and multidrug-efflux pumps severely limit the effectiveness of whole classes of antibiotics, the need is particularly pressing. An alternative approach to antimicrobial treatment is to use the well-characterized species-specific colicin-like bacteriocins which are produced by a wide range of Gram-negative bacteria, including Pseudomonas aeruginosa and Escherichia coli. Our current work on colicin-like bacteriocins aims to determine whether these potent antimicrobial agents are effective at killing bacteria growing in the biofilm state and during infection.

Bacterial biofilms and chronic infection

Antibiotic resistance is recognized as a major health concern and can be mainly attributed to the misuse and overuse of antimicrobial compounds [1]. One area where a lack of effective antibiotics is prominent is in the treatment of chronic bacterial infections, where bacteria appear to persist within the host and resist antibiotic therapy by means of specialized mechanisms, such as biofilm formation.

The biofilm is the most common growth mode adopted by bacteria and is now realized to be of great clinical significance [2]. A biofilm is a matrix-enclosed bacterial population in which bacteria adhere both to each other and also to surfaces or interfaces [3]. Although biofilm formation is known to be an important factor in numerous infectious diseases, their eradication from the host remains an extremely challenging task. Prominent chronic diseases in which bacterial biofilm formation enables persistence within the host and contributes to pathogenesis include chronic rhinosinusitis, bacterial endocarditis, Pseudomonas aeruginosa lung infection in patients with CF (cystic fibrosis), recurrent urinary tract infections and Crohn’s disease [4].

The formation of bacterial biofilms during the course of disease poses several difficulties for treatment. Within the biofilm, enclosed cells exhibit unique characteristics which cause increased resistance to antibiotics, generally between 10- and 1000-fold, relative to the planktonic state [5]. There have been several major reasons proposed to explain this phenotype including (i) slow or incomplete penetration of antibiotics due to the matrix barrier, (ii) presence of altered microenvironments within the biofilm including localized acidosis and anaerobiosis, (iii) induction of a resistant phenotype due to unique modes of gene expression, and (iv) the presence of an increased population of persister cells in biofilm populations that are effectively metabolically dormant cells that are highly resistant to antibiotics [5–8]. Although previous work has shown some significance in poor penetration and low oxygen concentrations for antibiotic efficacy, it is thought that the majority of biofilm-mediated antibiotic tolerance can be attributed to genetic factors, such as the production of periplasmic glucans in the biofilm state, and the presence of an increased persister cell population [6,8].

Colicin-like bacteriocins are highly specific and potent antibiotics

Bacteriocins are water-soluble protein toxins produced by a wide range of bacteria in response to nutrient stress and intra/inter-species competition. Colicins, which are produced by Escherichia coli, and other colicin-like bacteriocins, which are produced by a range of Gram-negative bacteria, kill bacteria closely related to the producing strain [9]. Colicins, which are the best characterized bacteriocins, are plasmid-encoded proteins, induced by DNA damage via the SOS response. These proteins contain three functional domains that mediate receptor binding, translocation across the cell envelope and cytotoxicity. On the basis of the mechanism of cytotoxicity, colicins can be divided into the enzymatic colicins, which exhibit nuclease function (against DNA, rRNA

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Abbreviations used: AIEC, adherent-invasive E. coli; CF, cystic fibrosis; SCV, small colony variant.

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or tRNA) or inhibit cell wall synthesis (through cleavage of lipid II), and the pore-forming colicins which depolarize the cytoplasmic membrane [10]. Colicin-producing strains also produce a specific immunity protein that protects the producing strain from the lethal effects of the protein toxin [11]. Colicin receptors on target cell membranes have been well characterized and are predominantly nutrient receptors. These include the BtuB receptor for vitamin B_{12} which is parasitized by colicins E1, E3 and E9 and the FhuA receptor for import of hydroxannate-type siderophores which is used by colicin M [12,13]. In addition, interactions with the TolB–ExbB–ExbD and TolABQR–Pal complexes, which mediate the translocation of group A and group B colicins across the cell envelope, have been extensively studied [14].

Similar to *E. coli*, the Gram-negative opportunistic pathogen *P. aeruginosa* produces colicin-like bacteriocins, termed the S-type pyocins. Like the colicins, production of these chromosomally encoded bacteriocins is induced by DNA-damaging agents [15]. The best characterized of the S-type pyocins are pyocins S1, S2 and S3, which kill cells through DNase activity, pyocin S4, which exhibits a tRNase activity, and pyocin S5, which is a pore-forming toxin [15,16]. The C-terminal domains of most pyocins show obvious homology with colicins that display the same killing activity. For example, colicin E9 and pyocin S2 possess homologous C-terminal DNase domains. Pyocins also have specific domains that mediate receptor binding and translocation, although, relative to the colicins, little is known about the mechanisms of pyocin uptake. However, the receptors of some pyocins have been identified, for example pyocin S2 and S4 both bind to the type I ferricyanoverdine receptor [16,17].

**Colicin-like bacteriocins show useful characteristics for the treatment or prevention of chronic bacterial infections**

The treatment of chronic biofilm-mediated bacterial infections is an area of large unmet clinical need. For example, chronic *P. aeruginosa* lung infection is the primary cause of mortality in CF patients [18]. Furthermore, recent findings now link a specific biofilm-forming *E. coli* pathotype, termed AIEC (adherent-invasive *E. coli*), with the chronic intestinal inflammation associated with Crohn’s disease [19,20].

A small number of recent studies suggest that colicin-like bacteriocins may be useful in the treatment and/or prevention of biofilm-mediated bacterial infections. For example, Trautner et al. [21] have shown that pre-growth of colicin-producing *E. coli* K-12 on catheters is able to prevent colonization by a colicin-susceptible *E. coli* clinical isolate, indicating that colicin production may act as a potent inhibitor of *E. coli* biofilm formation. Furthermore, colicin-producing *E. coli* strains have been shown to persist for longer periods in the murine gastrointestinal tract which is likely to involve inhibition of other enteric bacterial biofilms [22]. In addition, Saeidi et al. [23] have also suggested that pyocins could be utilized as anti-biofilm therapies. In this study, *E. coli* was utilized as a chassis organism and engineered to produce pyocins in response to its ‘sensing’ of *P. aeruginosa* [23]. The engineered *E. coli* was shown to sense and kill 99% of planktonic *P. aeruginosa* cells and also showed an ability to prevent the formation of *P. aeruginosa* biofilms [23]. Although suggestive that colicin-like bacteriocins may possess useful anti-biofilm activity, these studies do not directly test, in a quantitative manner, the ability of colicin-like bacteriocins to kill bacteria growing in the biofilm state. Our recent work has aimed to address this.

**Activity of pyocins against *P. aeruginosa* biofilms**

CF is the most common lethal hereditary disorder in Caucasian populations and is caused by mutations in the *CFTR* (cystic fibrosis conductance transmembrane regulator) gene, of which over 80 have been identified [18]. Disease predominantly presents itself in the lung, as mutations cause impaired electrolyte transport which leads to the accumulation of viscous and hyperosmolar mucus. Overall, this causes impaired eradication of inhaled microbes because of disrupted neutrophil and mucociliary clearance mechanisms [18]. In childhood, this altered lung environment usually leads to the development of acute, but severe, staphylococcal infections; however, as adulthood approaches, patients predominantly exhibit persistent *P. aeruginosa* infections [24,25]. In most patients with CF, chronic *P. aeruginosa* infection is the major cause of the airway damage that ultimately leads to death. It is thought that biofilm formation is a major factor in the ability of *P. aeruginosa* to persist in the lung and resist aggressive antibiotic therapy [25].

Given the role of *P. aeruginosa* in CF-associated lung infection, the development of novel antibiotics that are able to efficiently kill *P. aeruginosa* growing in the biofilm state would be of great clinical benefit. With this in mind, we have tested the ability of pyocins to kill *P. aeruginosa* growing in the biofilm state. In order to achieve this aim, mature biofilms of several *P. aeruginosa* clinical isolates were grown *in vitro* and subjected to treatment by pyocin S2 (in the form of the pyocin S2–Im2 complex) and the current frontline antibiotics tobramycin and aztreonam that are used for treatment of *P. aeruginosa* lung infection. Overall, the results showed that pyocin S2 exhibited superior killing activity against mature *P. aeruginosa* biofilms formed by phenotypically diverse strains, relative to both tobramycin and aztreonam [26]. Indeed, after antibiotic treatment (3 μg·ml^{-1}) of 24-h biofilms, survival of *P. aeruginosa* YIP7 was approximately 100-fold greater after aztreonam and tobramycin treatment, relative to treatment with pyocin S2 [26]. In addition, our most recent data show that pyocin S5 is also highly effective at killing a range of phenotypically diverse strains both in the planktonic and biofilm states (K. Smith and D. Walker, unpublished work).

A further complicating factor in the treatment of chronic *P. aeruginosa* lung infection is the appearance of antibiotic-resistant SCVs (small colony variants) that are
Can colicins be used for the treatment of AIEC infections associated with Crohn’s disease?

Crohn’s disease is a major form of inflammatory bowel disease which affects approximately 50 individuals in 100,000 in Europe and the U.S.A. [29]. It is characterized primarily by discontinuous, transmural and often granulomatous inflammation which can occur at all regions of the gastrointestinal tract [29]. Crohn’s disease is a highly debilitating disorder and usually comprises prolonged periods of remission followed by acute periods of active disease. There is currently no cure for Crohn’s disease, and, for most patients, permanent treatment can be found only in the form of invasive and severe surgery. The novel E. coli pathotype AIEC has been associated with the pathogenesis of Crohn’s disease [19,20]. AIEC have been shown to form thick biofilms on the ileal mucosa of approximately 30% of Crohn’s disease patients and adheres to and invades intestinal epithelial cells [30,31]. AIEC infection in Crohn’s disease patients is thought to be caused by microbial dysbiosis induced by intestinal inflammation. This was shown elegantly in a recent study by Craven et al. [32] in which murine models for intestinal inflammation exhibited significant gut microflora perturbation and also enable them to be used to selectively correct imbalances in the microbiome that are associated with chronic conditions such as Crohn’s disease.

In our initial work in this area, we tested the ability of colicin E1 to kill the AIEC strain LF82 when growing in the biofilm state. Mature LF82 biofilms were grown in vitro and treated with colicin E1 and the commonly used antibiotics ampicillin and ciprofloxacin. Our preliminary data indicates that colicin E1 exhibits superior killing against AIEC biofilms, relative to the small-molecule antibiotics tested (K. Smith and D. Walker, unpublished work).

Conclusions

Currently available antibiotics show poor efficacy against biofilm-mediated bacterial infections and this can be mostly attributed to the biofilm mode of growth which is characterized by persistent infection by resistant subpopulations. Our recent data indicate that colicin-like bacteriocins are highly effective at killing target strains growing in the biofilm state and so may make useful therapeutics for the treatment of chronic bacterial infections. In addition to their potency, the specificity exhibited by these protein antibiotics could enable them to be used to selectively correct imbalances in the microbiome that are associated with chronic conditions such as Crohn’s disease.

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