"STAMPS" SMART THERAPY AGAINST STREPTOCOCCUS MUTANS: REVIEW ARTICLE

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ABSTRACT

Streptococcus mutans has been implicated as the major acid producing cariogenic bacteria. Dietary sugars and other factors may cause an imbalance of oral micro flora that permits S. mutans to become dominant in the multi-species bio films on the tooth surface, which could lead to dental caries. The application of broad-spectrum antimicrobials often results in re-colonization, ecological disruption and re-dominance of S. mutans within oral flora resulting in secondary infections or other negative clinical consequences. To address this problem researchers have recently developed a new class of pathogen – selective molecules, called specifically (or selectively) targeted antimicrobial peptides (STAMPs), based on the fusion of a species - specific targeting peptide domain with a wide spectrum antimicrobial peptide domain.

Keywords: *Specifically targeted antimicrobial peptides, Streptococcus mutans.*

INTRODUCTION

Pathogenic microorganisms have been a constant source of human suffering and have urged the clinical development of novel therapeutics.[1,2,3] The conventional medical response to bacterial infections, administration of broad spectrum antibiotics, has become less effective against emerging pathogens due to the evolution of drug resistance stemming in part from the antibiotics abuse.[4] Additionally, antibiotics and oral antiseptics currently in use to treat mucosal infections eliminate pathogens and by stander bacteria alike, an outcome that can be associated with negative clinical consequences.[3,5]

Therefore, there is an unmet medical need to develop novel, narrow-spectrum therapeutics capable of maintaining the protective benefits of the normal micro flora during treatment.

Current approaches for treating dental caries and their possible limitations

Dental caries is one of the most prevalent and costly diseases throughout the world. Although the ultimate manifestation of the disease is the dissolution of tooth structure, the biological nature of the disease is a microbial infection caused primarily by the cariogenic bacterium mutans streptococci.[6] Caries arises from dietary sugars (primarily sucrose) that cause acidogenic microbes, such as S. mutans, to produce acids that damage tooth structure. This also leads to an imbalance in the oral micro flora that enables these cariogenic bacteria to become dominant in the multi-species bio films found on the tooth surface.[7,8]

The streptococci are the pioneer strains in plaque formation and mutans streptococci are the main etiological agent of dental plaque and caries.[9] During dental plaque formation, some oral bacteria are early colonizers that express biochemical components allowing them to adhere effectively to specific tissues (teeth or periodontal tissues). The later colonizers often contain components that facilitate them to adhere to the early colonizers, bringing competitive advantages. Within an established dental plaque, specific bacterial species are often found located adjacent to each other or mixed together to form unique structures that may confer adherence or growth advantages.[10]

Currently, most dental therapies are focused on eradicating the entire dental plaque via mechanical removal or broad spectrum antimicrobial treatments, while the only chemical intervention approved by FDA for caries prevention involve the use of fluoride-containing varnishes and toothpastes, which are capable of preventing enamel demineralization and reversing the process through remineralization[11,12]. Fluoride has been successful in reducing the incidence and prevalence of dental caries, but it has limited efficacy in killing cariogenic bacteria residing in dental plaque. This could be a factor contributing to the persistence of dental caries within populations, despite fluoride's well-documented clinical efficacy.

However the improved understanding of oral microbial ecology, especially the importance of the oral pathogens and commensally residents, has prompted interests in novel approaches focused on selective pathogen inhibition and modulation of the microbial composition of dental plaque to control pathogenesis.[6]

Rationale behind the specifically targeted antimicrobial peptides (STAMPs):

Antimicrobial peptides (AMPs) are among the repertoire of host innate immune defenses.[13,15] In the oral cavity, several AMPs are present in saliva and have antimicrobial activities against oral bacteria, including Streptococcus mutans, a primary etiologic agent of dental caries.[13,14,16] It is known that patients with "healthy" dental plaque displaying low levels of S. mutans are resistant to exogenous colonization from cariogenic pathogens and have shown long-term protection from dental caries.[10,17,18,19] Lack of S. mutans has been associated with sites of healthy dentition, while progressively increasing levels of S. mutans are associated with sites of caries development and cavitation.[20] Based on these findings, it was suggested that targeted antimicrobial therapies against S. mutans may be a good alternative approach to treat dental caries.[21]

Eckert et al. reasoned that, with the exception of a limited number of pathogens, the majority of indigenous oral microorganisms are benign or beneficial. Currently available antimicrobials exhibit broad spectrum killing properties resulting in indiscriminate killing of all microbes thereby disrupting the ecological balance of the indigenous micro biota with unknown clinical consequences.[10,22] These investigators formulated a new class of antimicrobials called Specifically Targeted Anti-Microbial Peptides (STAMPs).

STAMPs

A typical STAMP molecule consists of two functionally independent moieties conjoined in a linear peptide sequence: a non–specific antimicrobial peptide serves as the killing moiety while a species specific binding peptide comprises the target moiety. The target moiety provides the specific binding to a selected pathogen and facilitates the targeted delivery of the attached antimicrobial peptide.[10,22,23] STAMPs have shown effective elimination of S. mutans from a mixed species environment without affecting non – cariogenic oral streptococci.[6]

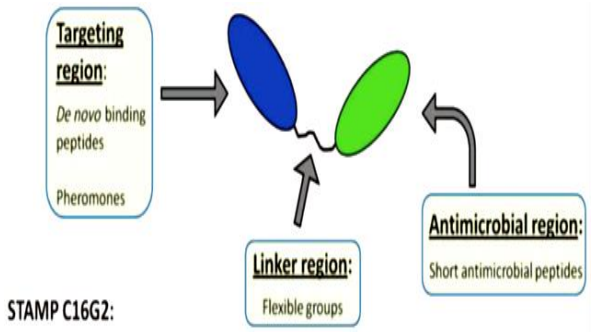

Fig. 1: Illustration of the basic structure for the specifically targeted antimicrobial peptides (STAMPs). Adapted from Eckert (2011)

Sources of STAMPs

Various derivatives from natural products, such as cranberry constituents, plant lectins, crude extracts of Morusalba leaves, beehives of honeybee (Apis mellifera)[24] and fractions of barley coffee have been shown to be effective against bio film formation of S. mutans. These substances can regulate the activities of surface anchored virulence factors glucosyl transferase and fructosyl transferase. Numerous small molecules including anthraquinones, apigenin, tt –farnesol, chitosan and 7 – epiclusianone, have been characterized and shown to have anti bio film activity towards S. mutans. However most of them were reported to lack selectivity against S. mutans bio films.

The prevention and treatment approaches based on these existing methods tend to disturb the ecological balance between pathogens and commensally residents in the oral cavity, which may lead to more severe infections. Therefore it is essential to develop a new approach which can selectively inhibit pathogenic bacteria and bio films. To accomplish this selectivity goal, pursuit of small molecules based upon nitrogen based marine alkaloids, have designed numerous derivatives of marine natural products based primarily on the 2–aminoimidazole (2-AI) scaffold and have shown that these compounds are potent inhibitors of bio film formation by both gram - positive and gram negative bacteria albeit the underlying mechanisms of bio film inhibition/dispersion is still under investigation.[25]

Antimicrobial Action

It has been hypothesized that the rapid bactericidal activity of such peptides is based on an primary binding to lipo polysaccharide (LPS) from gram negative bacteria, although the details of this mechanism appears to vary widely.[26] Previous observations have indicated the critical role of the general hydrophobic and cationic character of Anti-Microbial Peptides (AMP) function, including the significant contribution of aromatic tryptophan and cationic arginine residues found in many AMPs. Despite their small size (most are less than 50 amino acids), secondary structure also appears to play an important role in activity.

Certain linear AMPs can adopt an α – helical or β – strand confirmation upon interaction with hydrophobic environments such as deter gents or lipid vesicles that mimic bacterial membranes, suggesting that these conformational changes are necessary for antimicrobial function.[4]

Clinical Implications of STAMPs

The STAMP targeting region drives enhancement of antimicrobial activity by increasing binding to the surface of a targeted pathogen, utilizing specific determinants such as overall

membrane hydrophobicity, charge, and/or pheromone receptors, which in turn leads to increased selective accumulation of the killing moiety [1, 21,27]. As a result, STAMPs have increased killing potency, selectivity, and kinetics against targeted bacteria.[28]

Kreth et al., 2008 demonstrated that the sequence of inoculation deter mines whether cariogenic S. mutans or the health associated S. sanguinis compete or coexist with one another. Both species can persist in a bio film when inoculated at the same time, but given the chance to establish a bio film first, either species can preclude colonization of the other. STAMP technology is a potentially useful method for removing S. mutans from saliva, thus establishing an oral flora that can help prevent or reduce further infection of S.mutans.[6]

C16G2, a novel synthetic specifically targeted antimicrobial peptide with specificity for S. mutans consists of a S. mutanss elective 'targeting region' comprised of a fragment from S. mutans competence stimulation peptide (CSP) conjoined to a 'killing region' consisting of a broad spectrum antimicrobial peptide (G2).[28] Eckert et al., 2006, discovered that C16G2 was potent against S. mutans grown in a plank tonic or bio film state and did not affect other oral streptococci tested. C16G2 has rapid bactericidal activity against S. mutans, killing bacteria within seconds of contact, but is slower against untargeted bacteria, where several minutes to hours are required to reach similar levels of killing[22,30].

Multi-species bio films from which S. mutans has been eliminated by C16G2 resist re-colonization by S. mutans, demonstrating the protective colonization effect in vitro.[6] Mechanistic studies indicate that C16G2 is bactericidal via a cytoplasmic membrane disruption mechanism. Accumulation of C16G2 on the cell surface of S. mutans leads to loss of membrane potential, leakage of intracellular adenosine triphosphate (ATP), and loss of membrane integrity, followed by cell death.[30]

Jian et al generated a number of synthetic antimicrobial peptides against S. mutans via construction and screening of several structurally diverse peptide libraries with different biochemical characteristics. From these libraries, they identified multiple peptides with robust killing activity against S. mutans. To further improve their effectiveness, the most bactericidal peptides from each library were synthesized together as one molecule, in various combinations, with and without a flexible peptide linker between each antimicrobial region. They found that many of these "fusion" peptides had enhanced killing activities in comparison with those of the original nonconjoinedmolecules.[4]

A pilot study was conducted in humans to evaluate the efficacy of C16G2 in vivo by Sullivan et al., 2011. It was found that a single application of C16G2 in the oral cavity (formulated in a mouth rinse vehicle) was associated with a reduction in plaque and salivary S. mutans, lactic acid production, and enamel demineralization during the entire 4-day testing period. It has robust efficacy and selectivity for S. mutans, but no other oral bacteria, and affects targeted bacteria within seconds of contact.[29,31]

Other Choice of Agents and Approaches

In addition to 'traditional' agents, new approaches have been initiated to keep the oral bio film 'healthy'. Many efforts are devoted to extracts from plants and natural products. Also, photodynamic therapy has been developed, which combines a relatively inert dye that is photo activated and has bactericidal properties. Antimicrobial peptides in saliva and their synthetic analogues have promising potential.[32]

Replacement therapy has been researched for over 30 years, with cariogenic bacteria being modified to make them less acidogenic, while keeping their colonization resistance properties intact. Much of this work was started in the period when most attention was given to the arch criminal of caries, Streptococcus mutans.[32] Animal experiments involving lactate dehydrogenase deficient and ureolytic S. mutans strains have shown promising results in caries prevention. In the former case, the approach is directed against single microbial species. However one should not disregard the role of the other microorganisms, such as non mutan sacidogenic streptococci, in the caries process in humans.[6]

C16G2 is currently being developed by a Los Angeles-based biotechnology company, C3 Jian Inc. An Investigational New Drug (IND) application has been accepted by the US FDA, and the phase I clinical trial was started in the summer of 2012.[29]

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SUMMARY AND CONCLUSION

The STAMPs presented here are capable of eliminating S. mutans from multispecies bio films without affecting closely related non cariogenic oral streptococci, indicating the potential of these molecules to be developed into "probiotic" antibiotics which could selectively eliminate pathogens while preserving the protective benefits of a healthy normal flora. For each of these promising new approaches, the question remains how these agents can be made cost effective and how they can be formulated into usable products. This novel approach to fighting caries is still experimental and may be years away from clinical use. However, new technologies such as STAMP might hold the key to the improvement of oral health for millions of people.

It might also be the piece of the puzzle that will be used to treat a multitude of other diseases that

require the elimination of specific pathogens without the damage to the existing normal healthy flora.

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