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Quorum sensing inhibition - combating biofilm infections - A target to remedy

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Abstract

Periodontal disease is an inflammatory disease causing destruction of the supporting structures of the teeth. Micro-organisms can actively maintain their three-dimensional biofilm structure and lead to its progression. The severity of periodontal infection can be greatly affected by the ensuing host response. Increased synthesis of the signal molecules known as autoinducers by the pathogenic bacteria integrate with native signal-transduction systems to produce regulatory networks that coordinate gene expression and function as a decision-making process to regulate the production of virulence factors. Any mechanism that can effectively interfere with this bacterial cross-talk, could be used for quorum sensing inhibition and preventing microbial infections. Pharmacologic interference of intercellular signaling includes furanones, herbal products such as garlic, curcumin, citrus flavonoids, turmeric or L-canavanine. Use of such plant based molecules along with antibiotics may cause some benefit in combating the periodontal disease. The use of sophisticated drugs comprising both biofilm-controlling compounds and antibiotics can be used to treat periodontal infections.

Keywords: Biofilm, Autoinducers, Bacterial Cross-talk, Quorum Quenching, Furanones

Introduction

Periodontal disease is a consequence of ecologically driven imbalance of the oral microbial biofilm. When equilibrium in the plaque community is altered inflammation is induced by an increased flow of GCF, increased nutrients and pH rise, the growth of periodontal pathogens is favoured.[1] Structured consortium attached on a living or inert surface formed by the microbial cells attached to each other and surrounded by the self-produced extracellular polymeric matrix is known as biofilm.[2] Biofilm micro-organisms form distinct three dimensional structured communities with fluid channels for the transport of substrate, waste products and signal molecules.[3,4] Micro-organisms actively maintain their three-dimensional biofilm structure and prevent other micro-organisms from clogging the channel systems by releasing surfactant molecules.[5] Therefore, once a bacterial biofilm infection is established, it becomes difficult to eradicate.

Quorum sensing- bacterial cross-talk:

Gram-positive and Gram-negative bacteria coordinate their behavior through cell-to-cell communication mediated by small, diffusible signals.[6] Greenberg and colleagues

(1999) termed it as 'Quorum Sensing' based on coordinate behavior of a minimal unit of microorganisms.[6] Quorum sensing includes the production and release of small biochemical signal molecules by the bacterial cell, either actively or passively into the surrounding environment and recognition of these molecules by specific receptors leading to changes in gene regulation.[7]

Increased synthesis of the signal molecule creates a positive feedback cycle, which is why quorum signals are commonly called 'Autoinducers'.[7] Gram-positive bacteria use these autoinducers (AI) from two processes in quorum sensing that is: A two component signal transduction system and internalization.[8]

Among the Gram-negative bacteria, the most well studied quorum-sensing system is the LuxR-LuxI homologous system and the cognate N-acyl-homoserine lactones (AHL) signal molecules.[9] First identified in marine Vibrio species, it is involved in the regulation of many host associated phenotypes, including the production of virulence factors and secondary metabolites.[10-13] Lux-type systems consist of 2 components, an autoinducer synthase, which synthesizes AHL from S-adenyosyl methionine, and a transcriptional regulator.[7] As the population density increases, intracellular AHL binds the functionally linked cognate at a sufficient concentration that in turn modulates the expression of quorum sensing–regulated genes**.**(Fig 1) Once transferred to a new bacterial genome, these systems integrate with native signal-transduction systems to produce regulatory networks that are often unique to a given species.

In a study by Frias et al, culture media from periodontal pathogens such as F. nucleatum, P. gingivalis and P. intermedia induced bioluminescence in the reporter strain, suggesting that these organisms also produce AI 2.[14] DNA sequence analysis has confirmed the presence of highly conserved homologues of AI-2 synthase gene, luxS, in S. gordonii,[15] P. gingivalis and A. actinomycetemcomitans.[16] AI 2 produced by A. actinomycetemcomitans regulates the expression of virulence factors, biofilm formation, iron uptake and also upregulates leukotoxic activity and production of leukotoxin polypeptide.[17] P. gingivalis is known to adhere to the oral streptococci in a species specific manner and this may represent one mechanism by which P. gingivalis initially colonizes the dental biofilm.[18] Similar AI 2 mediated cross talk is possible between A. actinomycetemcomitans and P. gingivalis and the AI 2 signal of A. actinomycetemcomitans[15,16] is capable of modulating the expression of luxS regulated genes in P. gingivalis.

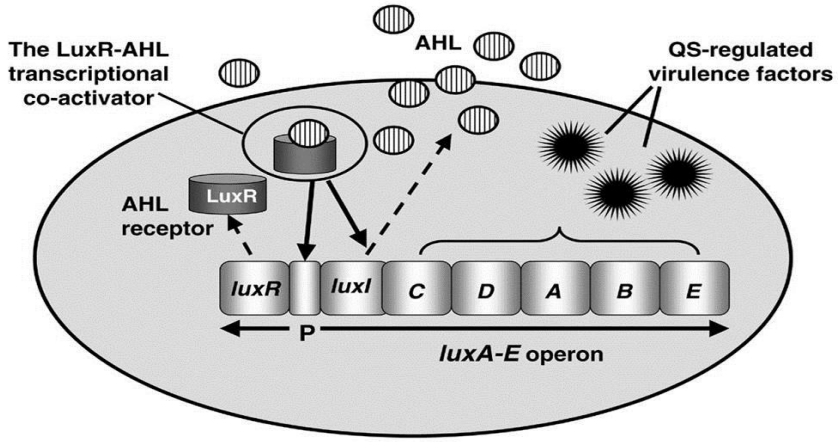

Fig. 1: Auto inducer type quorum sensing signal system in Gram-negative bacteria.

Quorum sensing inhibition:

At present, there is a general agreement that use of conventional mechanical therapy is the gold standard for the reduction in bacterial insult but use of chemotherapy has also gained importance in the past. However, the problem with this approach is that the bio-film formation restarts immediately after mechanical debridement. The rapid emergence of superbugs that resist the most commonly used antibiotics has emphasized the need for novel strategies against microbial pathogens.[19]Hence, for therapeutic purposes, it is necessary to attack the established biofilm.

For prophylactic purposes, it seems reasonable to target processes involved in the biofilm formation that have the potential to cause disease, without perturbing the balance of the normal flora.[20] The fact that quorum sensing is linked to virulence factor production suggests that many virulent Gram-negative organisms could be rendered nonpathogenic by inhibition of their quorum-sensing systems.[21]

Quorum quenching:

Any mechanism that can effectively interfere with any of the key processes in quorum sensing, could be potentially used for the inhibition of quorum sensing and preventing microbial infections. In the recent years, a number of groups of potent quorum-quenching chemicals and enzymes have been identified.[6] Pharmacologic interference of intercellular signaling can be envisioned at several steps in the quorum-sensing circuitry.[6] Brackman et al. demonstrated that QS inhibitors increased the susceptibilities of both Gram-positive and -negative bacterial biofilms to antibiotics in vitro and in vivo.[22]

Inhibition of AHL signal generation:

Knowledge about signal generation can be exploited to develop quorum-sensing inhibitor molecules that target AHL signal generation. Majority of the bacteria producing AHL signals encode one or more genes homologous to luxI of Vibrio fischeri. Various analogs of S-adenosyl methionine, such as S-adenosylhomocysteineand S-adenosylcysteinehave been demonstrated to be potent inhibitorsof AHL synthesis.[23] Some recent reports have demonstrated that certain macrolide antibiotics are capable of repressing AHL synthesis when applied at subminimal growth inhibitory concentrations. Macrolides are known for the inhibition of protein synthesis at the ribosomal level.[24]

Inhibition of AHL signal dissemination:

Bacterial cell-to-cell communication can be inhibited by a reduction in the active signal molecule concentration in the environment. Dong et al.[19] found a bacillus species that produced an enzyme that catalyzed the hydrolysis of AHL molecules. Another study[25] on quorum sensing isolated more than 20 bacteria belonging to Bacillus cereus group which were capable of enzymatic inactivation of AHLs.

Inhibition of AHL signal reception:

Quorum-sensing signal transduction can be blocked by an antagonist molecule capable of competing or interfering with the AHL signal for binding to the LuxR-type receptor. Competitive molecules invariably would be structurally similar to the native AHL signal, in order to bind to and occupy the AHL-binding site but fail to activate the LuxR-type receptor. Noncompetitive inhibitors however, may show little or no structural similarity to AHL signals, as these molecules bind to different sites on the receptor protein.[6]

It is known that higher organisms also interfere with AHL-mediated quorum sensing. A number of plants secrete substances that mimic bacterial AHL signal activities and quench quorum sensing–regulated behaviors. Exudates from

Pisumsativum were demonstrated to contain several separable activities that either stimulated or inhibited bacterial AHL-dependent phenotypes.[26] Several systems that disrupt homoserine lactone-mediated quorum-sensing systems are also found in nature, such as those produced by bacillus and variovorax species.[26]

The observation that fronds of the Australian red macroalgaDeliseapulchra are rarely polluted with marine biofilms led to the discovery of a class of halogenated 'Furanones' with quorum-sensing inhibitory activity.[26] D. pulchrafuranone compounds consist of a furan ring structure with a substituted acyl chain at the C-3 position and a bromine substitution at the C-4 position. An important discovery was that furanone compounds compete with the cognate AHL signal for the LuxR receptor site and repress AHL dependent expression, inhibit AHL controlled virulence factor production and inhibit quorum sensing–controlled virulence of E.carotovora.[27] One drawback, however, is that the halogenated furanone derivatives are probably too toxic for humans.

Side-effects related to toxicity can be avoided by using Herbal biofilm-controlling compounds.[28]Recently, the anti-biofilm properties of garlic, curcumin, citrus flavonoids, turmeric, grapefruit, Nutmeg, Clove extract and caffeine have also been investigated.[29]

L-canavanine, an arginine analog found in the seeds of legumes, can act as a quorumsensing inhibitor and holds promise for the manipulation of the quorum-sensing systems in other bacteria.[28]

The frequently used broad-spectrum anti-microbial agent, bis-phenol Triclosan, may also inhibit the synthesis of the homoserine lactones. The search for homoserine lactone-mediated quorum-sensing, however, has failed to identify this system in oral bacteria.[29]

Quorum quenching in periodontal research:

Physiological and biological aspects of quorum sensing have received considerable attention and have begun to be studied at a molecular level. The pathogenic behavior of periodonto-pathogenic bacteria like P. gingivalis, A. Actinomycetumcomitans, Streptococcus species is communicated and coordinated through quorum sensing. Biofilm formation in different bacteria occurs through various genes and pathways with various quorum sensing mechanisms. Various means of inhibiting quorum sensing along with mechanical plaque removal and oral hygiene measures may help to reduce the periodontal disease severity. Compounds that antagonize quorum sensors may potentially be useful in inhibiting the virulence mechanisms and biofilm formation. Research is being focused on the benefits of quorum sensing inhibition in addition to antibiotics.[29] Use of the plant based molecules along with antibiotics for quorum quenching may cause some benefit in combating the periodontal disease. Newer molecules that can inhibit the bacterial biofilm formation will be highly appreciated. All these new treatment modalities are certainly useful, but an adequate oral hygiene will always be the gold standard for periodontal health.[30]

Conclusion

Developing oral prophylactic strategies through interference of quorum sensing of biofilm microorganisms represents an interesting future challenge. Such approaches may interfere with microbial adaptive pathways without killing the microorganisms. The study of bacterial quorum sensing has suggested several ideal targets for drug design. Future biofilm infection treatment will probably use sophisticated drugs tailored to the causing pathogen.

Future perspectives

In a brief span of time, numerous quorum quenching phenomena have been observed and a number of quorum quenching strategies have been developed with promising results. The degradation of the quorum sensing signal itself is not sufficient to completely diminish the activities in a number of cases. Development of quorum quenching enzymes to target a broad range of signaling molecules is definitely a challenge. Further investigation should focus on search for the universal quorum quenching enzymes that target a number of AHLs for efficient blockade of quorum sensing activity. Use of proteomic and genomic strategies should help to elucidate the phenotypes associated with quorum sensing. Also, a combination of the quorum quenching approach with other treatments, such as antibiotics, to obtain a synergistic effect is a potential strategy. It is based on the observation that most quorum-quenching compounds enhance the sensitivity of pathogens to antibiotics, even if the quorum-quenchers could not achieve complete dissolution of the biofilms.

References

1. Marsh PD. Microbial ecology of dental plaque and its significance in health and disease. Adv Dent Res 1994; 8:263-271.
2. De Fuente-Nu ez C, Reffuveille F, Fernandez L et al. Bacterial biofilm development as a multicellular adaptation: antibiotic resistance and new therapeutic strategies. CurrOpinMicrobiol 2013; 16(5): 580–589.
3. Hall-Stoodley L, Costerton JW, Stoodley P. Bacterial biofilms: from the natural environment to infectious diseases. Nat Rev Microbiol 2004; 2(2): 95–108.
4. Costerton JW. Introduction to biofilm. Int J Antimicrob Agents 1999; 11:217-221.
5. Davey ME, O'Toole GA. Microbial biofilms: from ecology to molecular genetics. MicrobiolMolBiol Rev 2000; 64:847-867.
6. Hentzer M, Givskov M. Pharmacological inhibition of quorum sensing for the treatment of chronic bacterial infections. Journal of Clinical Investigation. 2003;112(9):1300-1307.
7. Costi D. Sifri. Quorum Sensing: Bacteria Talk Sense. Clin Infect Dis. 2008; 47 (8): 1070-1076
8. Redfield RJ. Is quorum sensing a side effect of diffusion sensing? Trends Microbiol 2002; 10:365–70.
9. Eberl, L. N-acyl homoserinelactone-mediated gene regulation in gram-negative bacteria. Syst. Appl. Microbiol. 1999; 22:493–506.
10. Passador, L., Cook, J.M., Gambello, M.J., Rust, L., and Iglewski, B.H. Expression of Pseudomonas aeruginosa virulence genes requires cellto- cell communication. Science. 1993; 260:1127–1130.
11. Von Bodman, S.B., and Farrand, S.K. Capsular polysaccharide biosynthesis and pathogenicity in Erwiniastewartii require induction by an N-acylhomoserine lactone autoinducer. J. Bacteriol.1995; 177:5000–5008.
12. Lewenza, S., Conway, B., Greenberg, E.P., and Sokol, P.A. Quorum sensing in Burkholderiacepacia: identification of the LuxRI homologs CepRI. J. Bacteriol. 1999; 181:748–756.
13. Latifi, A., et al. Multiple homologues of LuxR and LuxI control expression of virulence determinants and secondary metabolites through quorum sensing in Pseudomonas aeruginosa PAO1. Mol. Microbiol. 1995; 17:333–343.
14. Frias J, Olle E, Alsina M. Periodontal pathogens produce quorum sensing signal molecules. Infect Immun 2001;69:3431-4.

15. McNab R, Ford SK, El-Sabaeny A, Barbieri B, Cook GS, Lamont RJ. LuxS-based signaling in Streptococcus gordonii: autoinducer 2 controls carbohydrate metabolism and biofilm formation with Porphyromonasgingivalis. J Bacteriol 2003; 185:274-284.
16. Fong KP, Gao L, Demuth DR. LuxS and arcB control aerobic growth of Actinobacillusactinomycetemcomitans under iron limitation. Infect Immun 2003; 71:298-308.
17. Novak EA, Shao H, Daep CA, Demuth DR. Autoinducer-2 and QseC control biofi lm formation and in vivo virulence of Aggregatibacteractinomycetemcomitans . Infect Immun 2010;78:2919-26.
18. Shao H, Demuth DR. Quorum sensing regulation of biofilm growth and gene expression by oral bacteria and periodontal pathogens. Periodontol 2000 2010;52:53-67.
19. Dong Y-H, Wang L-H, Zhang L-H. Quorum-quenching microbial infections: mechanisms and implications. Philosophical Transactions of the Royal Society B: Biological Sciences. 2007;362(1483):1201-1211.
20. Hancock RE. Host defence (cationic) peptides: what is their future clinical potential? Drugs 1999; 57:469-473.
21. Payne DJ, Warren PV, Holmes DJ, Ji Y, Lonsdale JT. Bacterial fatty-acid biosynthesis: a genomics-driven target for antibacterial drug discovery. Drug Discov Today2001; 6:537544.
22. Brackman G, Cos P, Maes L et al. Quorum sensing inhibitors increase the susceptibility of bacterial biofilms to antibiotics in vitro and in vivo. Antimicrob Agents Chemother 2011; 55(6): 2655–2661.
23. Tateda, K., et al. Azithromycin inhibits quorum sensing in Pseudomonas aeruginosa. Antimicrob. Agents Chemother.2001; 45:1930–1933.
24. Pechere, J.C. Azithromycin reduces the production of virulence factors in Pseudomonas aeruginosa by inhibiting quorum sensing. Jpn. J. Antibiot.2001; 54:87–89.
25. Leadbetter, J.R., and Greenberg, E.P. Metabolism of acyl-homoserine lactone quorumsensing signals by Variovoraxparadoxus. J. Bacteriol. 2000; 182:6921–6926.
26. Swift S, Lynch MJ, Fish L, Kirke DF, Tomás JM, Stewart GS, et al. Quorum sensingdependent regulation and blockade of exoprotease production in Aeromonashydrophila. Infect Immun 1999;67:5192-9.
27. Hentzer M, Riedel K, Rasmussen TB, Heydorn A, Andersen JB, Parsek MR, et al. Inhibition of quorum sensing in Pseudomonas aeruginosa biofilm bacteria by a halogenated furanone compound. Microbiology 2002;148:87-102.
28. Estrela, A.B.; Abraham, W.-R. Combining Biofilm-Controlling Compounds and Antibiotics as a Promising New Way to Control Biofilm Infections. Pharmaceuticals 2010, 3, 1374-1393.
29. Whittaker CJ, Klier CM, Kolenbrander PE. Mechanisms of adhesion by oral bacteria. Annu Rev Microbiol 1996; 50:513-552.
30. S.Yadav.Quorum Sensing Inhibition, Relevance to Periodontics. Journal of International Oral Health 2015;7(1):1-3.