

Disruption of Bacterial cell-to-cell Communication (Quorum Sensing): A Promising Novel Way to Combat Bacteria-Mediated Diseases

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ÖZET

Bakteriyel iletişimin sisteminin (Quorum Sensing) kesilmesi: Bakteri aracılı hastalıklarla savaşta umut vaat eden yeni bir yol

Antibiyotikler bakteriyel enfeksiyonların tedavisinde yaygın bir şekilde kullanılmaktadırlar. İlaç tedavisinde antibiyotiklerin gereksiz ve rastgele kullanımları, bu ajanlara karşı yaygın direnç gelişmesine neden olmuştur. Çoklu antibiyotik direncine sahip bakterilerin ortaya çıkması ve hızla yayılması, halihazırda mevcut antibiyotiklerle bakteriyel enfeksiyonların tedavisini giderek güçleştirmektedir. Dolayısıyla, bakterilerle savaş yeteneğimizin azaldığı post-antibiyotik bir dönemle karşı karşıyayız ve enfeksiyöz hastalıklarla başa çıkmak için yeni stratejilere olan gereksinim gün geçtikçe artmaktadır. Patojenik bakterilerin patojenite ve virülans faktörlerinin üretimini bakteriler arası iletişim sistemini kullanarak düzenlediklerinin keşfi, bakteriler arası iletişim sistemini antimikrobiyal tedavi için cazip bir hedef haline getirmiştir. Bakterileri öldürmek yerine patogenezi hedef almak, direnç gelişimi için daha az seçici baskı uygulayabilir, bu nedenle bakteriyel iletişim inhibitörleri kullanarak bakteriler arası iletişimin inaktive edilmesi, enfeksiyöz hastalıkların tedavisinde büyük umut vaat etmektedir.

Anahtar sözcükler: Hücreler arası iletişim, antibiyotik direnci, quorum sensing inhibitörleri

ABSTRACT

Disruption of bacterial cell-to-cell communication (Quorum Sensing): a promising novel way to combat bacteria-mediated diseases

Antibiotics are commonly used for the treatment of bacterial infections. The inappropriate and indiscriminate application of antibiotics in pharmacotherapy has led to the development of widespread resistance to these agents. With the emergence and spread of multi antibiotic-resistant bacteria, it is becoming increasingly more difficult to treat bacterial infections with currently available antibiotics. Thus, we are facing a post-antibiotic era with a reduced capability to fight bacteria, and, hence, there is an increasing need for new strategies to cope with infectious diseases. The discovery that many pathogenic bacteria employ quorum sensing (QS) to regulate their pathogenicity and virulence factor production makes the QS system an attractive target for antimicrobial therapy. Targeting the pathogenesis instead of killing the organism may provide less selective pressure for the development of resistance. Therefore, it has been suggested that inactivating the QS system in bacteria by the use of QS inhibitors holds great promise for the treatment of infectious diseases.

Key words: Cell-to-cell communication, antibiotic resistance, quorum sensing inhibitors

INTRODUCTION

The discovery of antibiotics has been a milestone in the history of medicine. With the introduction of antibiotics into clinical practice, infectious diseases that were once untreatable have become treatable and millions of lives have been saved by taking many dangerous bacterial infections under control. However, this medical miracle is being deteriorated by the development and rapid spread of bacterial resistance (1). Today, a global concern has emerged that we are facing a post antibiotic era with a limited

capability to fight bacterial infections (2). The increasing occurrence of multiantibiotic-resistant pathogen strains has gradually made conventional antimicrobial treatment ineffective. Thus, there is a great need to develop novel strategies to combat microbes without selecting for resistance. The discovery that many pathogenic bacteria employ bacterial cell-to-cell communication or quorum sensing (QS) system to regulate their pathogenicity and virulence factor production makes this system a novel therapeutic target for the design and development of a new class of drugs that potentially control pathogenicity and

attenuate virulence. Compounds capable of reducing bacterial virulence are termed antipathogenic drugs. Antipathogenic drugs target key regulatory mechanisms that control the expression of virulence factors in pathogenic bacteria and can render pathogenic bacteria non-virulent (3). In fact, this approach has already shown promise in the battle against human pathogen *P. aeruginosa* (2).

Quorum Sensing

QS is a cell-to-cell communication system employed by a variety of Gram (-) and Gram (+) bacteria to coordinate group behaviors as function of cell-density. QS depends on the production of small signal molecules which enable a bacterium to monitor its own cell-density and coordinate critical gene expression (4). QS system was first described in the 1970s in the Gram (-) marine luminescent bacterium *Vibrio fischeri* (5). Long thought to be unique to *Vibrio fischeri* and certain closely related marine bioluminescent bacteria, it is now widely recognized that a variety of pathogenic bacteria use QS to control several processes including virulence factor production, antibiotic production, motility and the formation of biofilm (2,6). By perceiving the signaling molecules, bacteria can modulate above mentioned processes in a cell-density dependent manner. For example, in a bacterial infection, bacteria avoid producing and releasing virulence factors until they reach a critical population density and, by doing so, they ensure

that they become numerous enough to overwhelm the host's immune system (2). Several types of signaling molecules have been identified in the last two decades (Figure 1). N-acylated-L-homoserine lactones (AHLs) are the most common class of signaling molecules in Gram (-) bacteria, while autoinducing peptides (AIPs) are the major class of signaling molecules in Gram (+) bacteria. Autoinducer-2 (AI-2) is a third category of signaling molecules that is employed by both Gram (-) and Gram (+) bacteria and is believed to be used for intra- and inter-species communication (7). In this paper, the current state of research concerning inhibition of acyl HSL-mediated QS in Gram (-) bacteria is reviewed.

Ahl-Mediated Quorum Sensing

Among QS signaling molecules, the most intensely studied signals are AHLs of Gram (-) bacteria. AHLs signalling was first described in *Vibrio fischeri* and has become a model for studies of QS. In many Gram (-) bacteria, AHL-based QS essentially involves the same series of events. The AHL

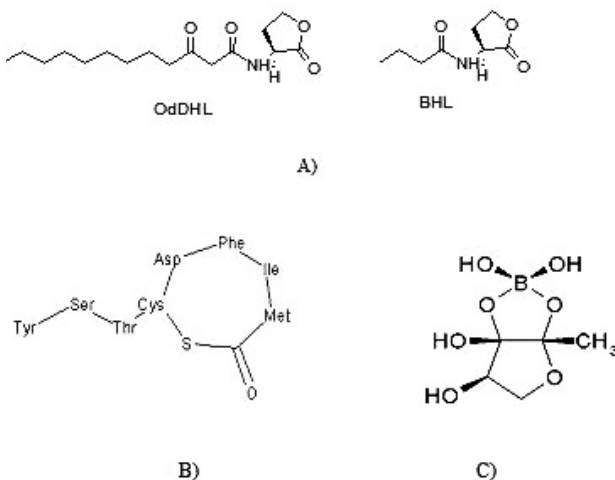


Figure 1: QS signaling molecules utilized by various bacteria. A) AHLs employed by Gram (-) bacteria. B) Oligopeptide employed by Gram (+) bacteria. C) Autoinducer-2 (AI-2) employed by both by Gram (-) and Gram (+) bacteria.

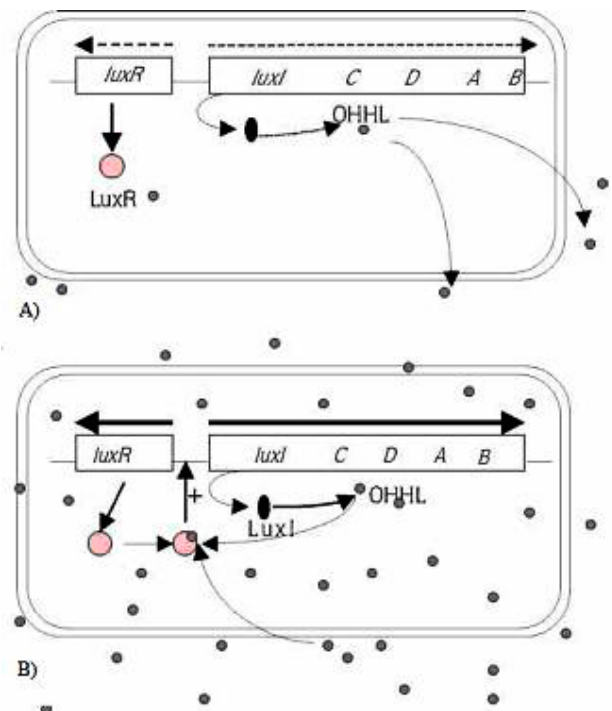


Figure 2: The regulation of bioluminescence in *V. fischeri* A) At low population density, *luxR* and *luxI* are transcribed at a low level and there is insufficient accumulation of OHHL. B) When the intracellular concentration of OHHL reaches a certain threshold, an OHHL-LuxR complex activates transcription of the *luxICDABE* operon resulting increased levels of OHHL and bioluminescence (8).

signal is synthesized at low basal level by an enzyme (a LuxI-type synthase). AHL signals diffuse out from bacterial cells and accumulate in the medium as a function of cell growth. At a threshold population density, the accumulated AHLs interact with a transcriptional activator protein (a LuxR-type transcriptional regulator) which will then induce the expression of QS-regulated genes (Figure 2) (8). AHL-mediated QS systems have been identified in human pathogens such as *Pseudomonas aeruginosa* (9), *Yersinia pseudotuberculosis* (10), *Burkholderia cepacia* (11), and *Escherichia coli* (12).

Quorum Sensing as a Target for Antimicrobial Therapy

Conventional antibiotics work by either killing (bactericidal) or inhibiting the growth of bacteria (bacteriostatic). However, this imposes hard the selective pressure for the development of antibiotic resistance. Therefore, alternative strategies to combat bacterial infections are needed more than ever. It has been observed that disrupting the QS system of a pathogen can result in a significant decrease in virulence factor production. For example, in human pathogen *P. aeruginosa*, QS is involved in the production of extracellular virulence factors such as elastase, protease, hemolysins, exotoxin A, rhamnolipid, pyocyanin, pyoverdinin and formation of biofilms and their tolerance to antimicrobial agents (2,13-15). Importance of QS to establish a successful infection has been shown in number of different infection models such as mouse burn wound, pulmonary infection and keratitis by employing QS deficient strains (16,17). In these studies, inadequacy of QS deficient strains to establish successful infection was proposed to be associated with reduced production of virulence factors. In addition to virulence factor production, many pathogenic bacteria such as *P. aeruginosa* also employ QS to control the formation of biofilms (18,19). It has been estimated that bacterial biofilms are responsible for over 80% of human infections (19-21). Biofilm-associated infections are very difficult to treat with traditional antibiotics as bacteria are embedded in an extracellular matrix composed of exopolysaccharides and proteins that reduce the contact between bacteria and antibacterial agent (21). Targeting the QS systems of pathogens is a novel plan of attack. Inhibitors and antagonists of bacterial QS

might constitute a new generation of antimicrobial agents. Blocking of QS in bacteria by the use of QS inhibitors (antipathogenic drugs) may find applications in medicine, agriculture and food technology (2,22). The QS system can be interfered with in a number of ways, including (1) inhibition of AHL molecule biosynthesis, (2) degradation of AHL molecules by bacterial lactonases and (3) using small molecules to block the activation of AHL receptor protein (22,23).

QS Inhibitors

Up to date, various types of screening have been carried out to find potential QS-inhibitory compounds. The first demonstration of QS inhibitors came from the Australian red macro alga *Delisea pulchra*. *D. pulchra* was found to produce a cocktail of halogenated furanone compounds some of which were able to inhibit QS. It was suggested that halogenated furanones inhibit AHL-mediated gene expression by competitively binding to the AHL receptor site on the LuxR-type protein. Gram et al. (24) demonstrated the potential of pure samples of furanones in preventing swarming motility in *Proteus mirabilis* at concentrations that did not effect bacterial growth. The natural *D. pulchra* furanone compounds are unable to inhibit human pathogen *P. aeruginosa* QS systems. However, synthetic furanone derivatives showed significant anti-QS activity in *P. aeruginosa* (2,25). Natural or synthetic furanone compounds were also found to inhibit a range of QS regulated phenotypes in a variety of bacterial species including *E. coli* O157:H7 and *Salmonella enterica* serovar *Typhimurium* and *Serratia liquefaciens* (26,27). These results suggest that furanone compounds could serve as a source to develop furanone-based drugs to block quorum-sensing systems in many Gram (-) bacteria. In addition to furanones, a number of other compounds that modulate AHL-mediated QS in Gram (-) bacteria have been identified. A wide range of natural substances, particularly extracts from plants and fungi have been shown to modulate AHL-regulated phenotypes in Gram (-) bacteria. Plants like garlic, water lily, pea seedlings, vanilla, *Medicago sativa*, extracts of *Tremella fuciformis*, *Panax ginseng*, *Scorzonera sandrasica*, extracts of various medicinal plants from South Florida, clove oil and tannic acid are known to modulate AHL-mediated QS

systems (15,22,23,28-30). Yang et al. (29) discovered that chlorzoxazone, salicylic acid and nifuroxazide were capable of interfering with QS in *P. aeruginosa*. A range of recognized drugs have been shown to have quorum sensing inhibitory activities. For example, some macrolide and nonmacrolide antibiotics have been shown to have effects upon AHL-mediated QS in Gram-negative bacteria. Skindorsoe et al. (31) identified that ceftazidime and iprofloxacin reduced QS-regulated gene expression in *P. aeruginosa*. The sub-inhibitory concentration of antibiotic tobramycin has been reported to reduce elastase production in *P. aeruginosa* (32).

CONCLUSIONS

The extensive usage of antibiotics in pharmacotherapy has led to the development of widespread bacterial resistance to these agents. Hence, there is a rising need to find alternatives to currently available antibiotics. The

disruption of QS systems of pathogenic bacteria has been regarded as the novel way to combat bacterial diseases. Inhibition of bacterial QS, rather than bactericidal or bacteriostatic strategies, offers a promising way to tackle the bacterial resistance problem. Because QS is not directly involved in processes essential for growth of bacteria, inhibition of QS should not impose harsh selective pressure for the development of resistance as with antibiotics. In addition, compounds that are capable of disrupting bacterial QS could potentially be used in combination with conventional antibiotics to increase the efficiency of disease control and to extend the life span of current antimicrobials. Over the course of the last 15 years, a large number of structurally diverse QS inhibitors have been discovered. Of the numerous molecules able to inhibit QS *in vitro*, a few have been successfully tested *in vivo* in animal models. Future work will reveal the possibility of using these QS inhibitors to treat bacterial infections in animals and humans.

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