Bioorganic Chemistry xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

The art of antibacterial warfare: Deception through interference with quorum sensing–mediated communication

Giordano Rampioni [a], Livia Leoni [a], Paul Williams [b,*]

[a] Department of Sciences, University Roma Tre, Viale Marconi 446, 00146 Rome, Italy
[b] Centre for Biomolecular Sciences and School of Life Sciences, University of Nottingham, University Park, Nottingham NG7 2RD, UK

ARTICLE INFO

Article history:
Available online xxxx

Keywords:
Quorum sensing inhibitors
Quorum quenching
Antivirulence drugs
New therapeutic strategies
Pathogenesis
Biofilm
Virulence factors

ABSTRACT

Almost a century on from the discovery of penicillin, the war against bacterial infection still rages compounded by the emergence of strains resistant to virtually every clinically approved antibiotic and the dearth of new antibacterial agents entering the clinic. Consequently there is renewed interest in drugs which attenuate virulence rather than bacterial growth. Since the metaphors of warfare are often used to describe the battle between pathogen and host, we will describe in such a context, the molecular communication (quorum sensing) mechanisms used by bacteria to co-ordinate virulence at the population level. Recent progress in exploiting this information through the design of anti-virulence deception strategies that disrupt quorum sensing through signal molecule inactivation, inhibition of signal molecule biosynthesis or the blockade of signal transduction and their advantages and disadvantages are considered.

1. Introduction

Infection is essentially a war between host and pathogen that, in the context of bacterial infections, was once thought to have been won through the discovery and development of diverse clinically effective broad spectrum antibiotics. However, the current antibiotic armamentarium has lost its effectiveness as a consequence of antibiotic resistance, the emergence of multi-antibiotic resistant bacteria and the difficulties of treating chronic, biofilm-centred infections. Conventional antibiotics either kill bacterial cells or prevent bacterial growth by targeting essential biochemical processes including cell wall, protein and nucleic biosynthesis. This in turn exerts enormous selective pressures leading to the evolution of antibiotic resistance. Further erosion of the antibiotic armamentarium has occurred because the development of new antibiotic classes has lagged far behind the requirement for such new drugs. Indeed no new antimicrobials acting against novel targets have entered late stage clinical trials in recent years [1]. Consequently, there is an urgent need to consider alternative strategies likely to lead to the development of clinically useful antibacterial agents particularly in this age of 'personalized medicine'.

Since the metaphors of warfare have been used extensively to describe the pathogenesis of bacterial infections, it is instructive to reflect on "The Art of War", a seminal work on military strategy and tactics written in the sixth century B.C. by the Chinese General Sun Tzu [2]. In fact he could well have been writing about the fate of bacterial pathogens facing new growth inhibitory antibiotics when stating, *"Confront them with annihilation, and they will then survive; plunge them into a deadly situation, and they will then live"*.

The urgent need for new therapeutic approaches to treat or prevent infections caused by antibiotic resistant bacteria has stimulated research toward the discovery and development of "anti-virulence" or "anti-pathogenic" drugs. Although bacterial growth inhibition *in vitro* may require bactericidal/bacteriostatic agents, this is not necessarily the case *in vivo*. Adaptation to growth in host tissues presents the infecting bacterium with a very different set of environmental challenges. Consequently bacteria have evolved multiple virulence determinants and the ability to form biofilms that cause host damage and disease. These in turn are controlled via sophisticated regulatory mechanisms. Consequently antibacterial agents which block colonization, interfere with metabolism or attenuate virulence factors or virulence gene expression without affecting bacterial growth *in vitro* offer potential advantages. These include expanding the repertoire of drug targets, preserving the host endogenous microbiome and exerting reduced selective pressures so delaying the emergence of resistance [3,4]. In other words, anti-virulence drugs should not *"confront... pathogens... with annihilation"*, but disarm them and overthrow their defences, so that the host can clear the infection.

The development of anti-virulence compounds requires a detailed understanding of the molecular mechanisms involved in

* Corresponding author. Fax: +44 (0)115 8467951.
E-mail address: paul.williams@nottingham.ac.uk (P. Williams).

http://dx.doi.org/10.1016/j.bioorg.2014.04.005
0045-2068/

host colonization and disease progression if they are to be exploited as potential therapeutic targets. As Sun Tzu wrote, *"What is of supreme importance in war is to attack the enemy's strategy"* [2]. The 'attack and destroy' strategy of most pathogens involves the production of adhesins to facilitate attachment to host tissues, followed by invasion of, or biofilm formation on, host cells/tissues either of which helps to protect the growing bacterial population from the host. These colonization activities are often followed by the deployment of exotoxins and tissue-degrading enzymes for combating host immune defences and the release of nutrients to 'feed' and expand the infecting bacterial 'army'.

Examples of anti-virulence compounds include inhibitors of bacterial attachment such as the 'pilicides', a family of bicyclic 2-pyridones (Fig. 1) which selectively disrupt a protein–protein interaction essential for the biogenesis of P-pili [4]. These mediate the attachment of *Escherichia coli* to bladder epithelial cells, an important stage in the development of urinary tract infections. A number of bacterial pathogens including the causal agents of typhoid fever and plague all utilize a virulence strategy involving the direct injection of proteins into human cells via a type III secretion system. High throughput screens have yielded compounds such as 2,2′-thiobis-(4-methylphenol) (Fig. 1) which is capable of inhibiting type III secretion in both *Yersinia* species and *Pseudomonas aeruginosa* [5]. In *Vibrio cholerae*, the causative agent of cholera, virstatin (4-(*N*-(1,8-naphthalimide))-n-butyric acid) (Fig. 1), blocks dimerization of the transcriptional regulator protein ToxT and so abrogates expression of the two main virulence determinants, cholera toxin and the toxin co-regulated pilus [4]. For most pathogens virulence is both multifactorial and combinatorial. In these cases one promising strategy is disruption of the "operations centre" i.e. global control systems such as quorum sensing that regulate the expression of multiple virulence determinants.

2. Quorum sensing as a therapeutic target

Quorum sensing (QS) is a cell-to-cell communication pathway that enables bacterial populations to co-ordinately re-programme gene expression in response to cell density. Briefly, in all QS systems, a signal molecule is produced and secreted (or freely diffuses) into the surrounding environment. As the bacterial population grows, the concentration of signal molecule(s) increases, until it reaches a threshold concentration at which it binds to and activates a cognate receptor protein. The perception of the QS signal molecule *via* the QS receptor triggers a physiological response in all members of the population, ultimately re-programming gene expression throughout the population. Therefore, through QS bacterial populations can modify their nature and dynamics, and act as a community to accomplish tasks that would be impossible to achieve for individual bacterial cells [6]. To defeat the enemy as Sun Tzu recommended, "*If united, separate them*" [2].

QS regulates a wide variety of physiological processes including bioluminescence, competence, antibiotic biosynthesis, motility, plasmid conjugal transfer, biofilm maturation, and the expression of key virulence factors in plant, animal and human pathogens belonging to diverse bacterial genera [6]. Indeed, many pathogens display markedly reduced virulence in infection models when their QS systems are disrupted by mutagenesis. QS also impacts on antibiotic susceptibility, either by increasing antibiotic tolerance in biofilms [7], by directly regulating antibiotic resistance genes such as *mecA* which confers methicillin resistance on *Staphylococcus aureus* [8], or by controlling the acquisition of antibiotic resistance genes by natural transformation as observed in *Streptococcus pneumoniae* [9]. Thus inhibiting QS may not only reduce virulence but also restore susceptibility to conventional antimicrobials. After all, "*In battle, there are not more than two methods of attack—the direct and the indirect; yet these two in combination give rise to an endless series of manoeuvers*" [2]. As Sun Tzu wrote, "*In the practical art of war, the best thing of all is to take the enemy's country whole and intact: to shatter and destroy it is not so good*" [2]. Consequently QS is considered a promising target for new anti-virulence agents.

Any anti-virulence strategy that is directed towards disruption of QS is commonly referred to as quorum quenching (QQ). Blocking communications within the opponent army has long been a major military tactic aimed at disrupting all possible co-operative activities. Sun Tzu warned, *"If words of command are not clear and distinct, if orders are not thoroughly understood, then the general is to blame"* [2]. A successful QQ strategy requires an in depth knowledge of the specific molecular actors and architecture of the QS system to be targeted. Indeed, "*The soldier works out his victory in relation to the foe whom he is facing*", and "*The opportunity of defeating the enemy is provided by the enemy himself*" [2].

Irrespective of their chemical and structural diversity, all QS systems reflect the classical scheme for bacterial cell-to-cell communication, in which the structure of the signal molecule contains information that is directed by a "sender" cell/organism to a "receiver" cell/organism. This common architecture provides multiple molecular targets for the action of enzymes or compounds interfering with QS-mediated cell-to-cell communication, namely (i) the biosynthesis of the signal molecule by the "sender" cell, (ii) the functionality and availability of the signal itself, and (iii) the reception/decoding of the message contained in signal molecule by the "receiver" cell (Fig. 2). As Sun Tzu stated, "*All warfare is based on deception*" [2]. Since targeting any of the three steps noted above would render bacterial cells incapable of perceiving their population size, and hence accomplishing QS-controlled tasks, it is evident that as an anti-virulence strategy, QQ is based on deception.

In the following sections, the molecular mechanisms underlying some of the best understood QS systems will be briefly described in the context of QS inhibition. Although, the development of potent, clinically effective QS inhibitors (QSIs) could have a significant impact on human health, there are also widespread opportunities

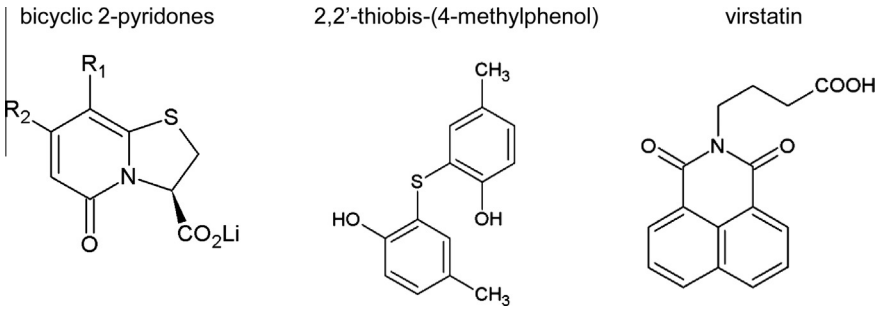

Fig. 1. Structures of exemplar anti-virulence agents which target attachment (pilicides), type III secretion (2,2′-thiobis-(4-methylphenol) and virulence gene regulation (virstatin) respectively.

Please cite this article in press as: G. Rampioni et al., Bioorg. Chem. (2014), http://dx.doi.org/10.1016/j.bioorg.2014.04.005