International Journal of Pharmaceutics xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Control of biofilm-associated infections by signaling molecules and nanoparticles

Alina Maria Holban[a,b,c], Monica Cartelle Gestal[d], Alexandru Mihai Grumezescu[b,*]

[a] Microbiology and Immunology Department, Faculty of Biology, University of Bucharest, Bucharest, Romania
[b] Department of Science and Engineering of Oxide Materials and Nanomaterials, Faculty of Applied Chemistry and Materials Science, University Politehnica of Bucharest, Bucharest, Romania
[c] Research Institute of the University of Bucharest, Bucharest, Romania
[d] Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia Athens, USA

ARTICLE INFO

Article history:
Received 10 December 2015
Received in revised form 26 February 2016
Accepted 27 February 2016
Available online xxx

Keywords:
Antimicrobial nanoparticles
Virulence modulators
Signaling molecules
Functionalized nanomaterials
Biofilm control

ABSTRACT

As the severe infections caused by resistant pathogens and biofilm embedded bacteria continue to emerge, alternative antimicrobial strategies could represent a solution. Recent studies support the development of molecular approaches (through signaling molecules) aiming to fight infections by modulating the virulence, behavior and formation of resistance structures such as biofilms. The utilization of such formulations would offer the advantage of reducing the selection of resistant isolates, since most of the proposed molecules do not interfere with the population fitness if utilized in low amounts. Despite the promising results, these therapies are delaying to be applied in the clinical context mainly because of the following: (i) limited knowledge regarding their long and medium term effect, (ii) specific properties that make most of these molecules difficult to be utilized in pharmacological formulations, (iii) low stability, (iv) difficulty to reach a target within the host body, and (v) limited availability. For reducing most of these disadvantages, nanotechnology seem to offer the best option through the development of nanostructured materials and nanoparticles able to improve the efficiency of molecular virulence modulators and novel antimicrobial compounds and to ensure their targeted delivery and controlled release.

1. Introduction

Similar to superior organisms, bacteria are able to communicate each other, using small, diffusible signals, which may act as autoinducers and are generically called Quorum Sensing (QS) and Response molecules (Hughes and Sperandio, 2008; Holban et al., 2014a, 2014d). The production of these signaling molecules is strictly controlled by some intricate molecular mechanisms which modulate the main microbial activities, such as virulence, biofilm formation and social behavior. This molecular communication may occur between bacteria belonging to the same species or to different species, but also between bacteria and their superior hosts (Holban and Lazăr, 2011). The molecular signals can either be cell-density related (and they are mainly referred to as autoinducers) or be produced by bacteria at different stages of growth, and they allow bacteria to monitor their environment and alter gene expression to derive a competitive advantage. The properties of these signals and the response they elicit are very important in ensuring bacterial survival and propagation in natural environments where hundreds of bacterial species coexist, being also important for pathogenesis (Jayaraman and Wood, 2008).

Recent reports bring increasing knowledge supporting the hypothesis that QS signaling represents the core regulatory system in bacteria and its manipulation could be the answer in the control of pathogenic microorganisms. Furthermore, the fact that virtually all bacteria phenotypes may be controlled by the right molecular cocktail brings new insights not only in the infection control but also in different industries and technologies aiming to take advantages of some beneficial traits of microorganisms (Holban et al., 2014a; Kjelleberg and Molin, 2002).

2. Molecular signaling in bacteria

The fact that bacteria can communicate each-other and with the host cells through small signalingmolecules represent a very intriguing and investigated aspect. Since this molecular communication seems to control all key virulence phenotypes and social behavior of pathogens it are currently considered important

* Corresponding author.
E-mail address: grumezescu@yahoo.com (A.M. Grumezescu).

http://dx.doi.org/10.1016/j.ijpharm.2016.02.044
0378-5173/

targets for the development of additional antimicrobial strategies (Holban et al., 2016a). The diversity of molecules and pathways involved in intra- and inter-species communication in bacteria and also between bacteria and host are schematically represented in Fig. 1.

2.1. Quorum sensing signaling in Gram Negative bacteria

N-Acyl Homoserine Lactones (AHLs) are the most common quorum molecules in Gram-negative bacteria. The QS is most commonly based ontwo component systems, composed by an effector and an receptor (LuxI and LuxR), based on the response proteins for the detection of the peptide autoinducer and a phosphorylation/dephosphorylation cascade that regulates the two component systems. Different AHLs vary in terms of acyl chain length and saturation state, as well as oxidation status at third carbon position. The homoserine lactone ring is always invariable and it is linked to the lactone chain via an amide bond. QS molecules are membrane permeable and they accumulate in both the intracellular and extracellular environment. Above threshold concentration, AHLs binds to cognate receptors to form activated complexes. AHL autoinducers also impact on interspecies signaling (Tay and Yew, 2013a; Holban et al., 2013a).

Autoinducer-2 (AI-2) represents another QS signaling molecule, probably implicated in interspecies signaling. This autoinducer it is also known for itsrole in the metabolism and bacterial fitness. AI-2 related signaling is present in many Gram negative bacteria but also in several Gram positives. However, the identification and categorization of AI-2 receptors is quiet limited to some bacterial species (Tay and Yew, 2013b).

The autoinducer 3 (AI-3)QS signaling was intensively studied in enteric microbiota. Studies reported that QseC/B two component regulatory system is documented as the sensor for AI-3 even though the structure and gene responsible for AI-3 production remain unknown. Inter-kingdom cross-talk studies revealed that catecholamine stress hormones epinephrine and norepinephrine are able to activate the QseC/B system; hence, it is possiblethat AI-3 resemble these hormones. Currently, AI-3 signaling has been described in *E. coli* and *S. typhimurium* (Pacheco and Sperandio, 2009a; Sperandio et al., 2003).

Other pathways include the 2-heptyl-3-hydroxy-4(1H)-quinolone (Pseudomonas Quinolone Signal [PQS]), 3-hydroxytridecan-4-one (cholera autoinducer-1 [CAI-1]) and *cis*-11-methyl-2-dodecenoic acid (diffusible signal factor [DSF]). The PQS pathway in *Pseudomonas aeruginosa*depends onthe operon *pqsABCDE* that controls the initial production of the PQS precursor, 2-heptyl-4 (1H)-hydroxyquinolone (HHQ). This operon is also controlling the subsequent oxidative hydroxylation reaction performed by the enzyme PqsH to convert HHQ to PQS. PQS is used only by *P. aeruginosa* for QS but HHQ may be utilized by other species belonging tothe Pseudomonas family and some of *Burkholderia* species. Both quorum sensing molecules interact with the transcriptional regulator, PqsR, to mediate virulence gene expression (Bala et al., 2014; Zhang et al., 2013). The production of Diffusible Signal Factor (DFS) described in *Xanthomonas campestris* pathovar *campestris* is dependent on the enoyl-CoA hydratase-related protein – RpfFand RpfB, a long-chain fatty acyl CoA ligase. In this two component signaling model, RpfC functions as the receptor and RpfG (which acts as an activated regulator component) is able to degrade the second messenger molecule cyclic di-GMP. This results in the modulationof virulence factors production and motility. Recent studies have identified *rpf* gene cluster homologs in others species such as *Xylella fastidiosa* or *Stenotrophomonas maltophilia*. Although the relevance of having multiple DSFs in one organism is still undefined, it is believed to be involved in interspecies or interspecies communication (Tay and Yew, 2013c; Holban et al., 2015; Caserta et al., 2014).

2.2. Quorum sensing signaling in Gram positive bacteria

Most of the QS systems of Gram positive bacteria are also based on the Lux system, or specific Two Component Systems (TCSs). Typical autoinducers in Gram positive bacteria are represented by small peptides named autoinducing peptides (AIPs). In a Gram-positive system, the peptide autoinducers increase in concentration with increasing cell density. The secreted peptide signals are detected by two component sensor kinases in the genetic circuit. The interaction of the peptide ligand induces a phosphorylation cascade that results in the phosphorylation of a cognate response regulator protein. This activates the response regulator facilitating DNA binding and hence altering the transcription of target genes involved in quorum sensing (Jimenez and Federle, 2014). Many gram-positive bacteria communicate with multiple peptides in combination with other types of quorum-sensing signals. QS signaling in Gram-positive bacteria operates through the activity of post-translationally modified oligopeptides, named auto-inducing

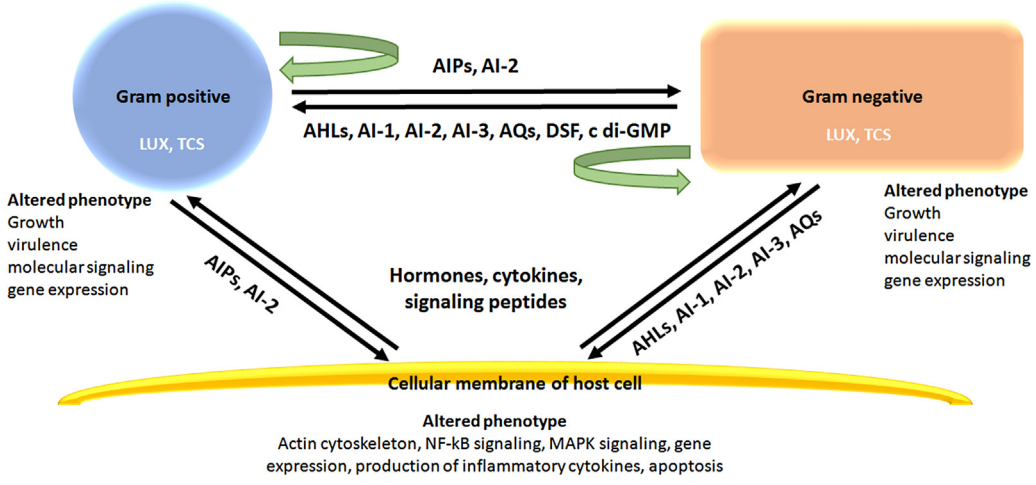

Fig. 1. Schematic representation of main signaling mechanisms and molecules involved in inter- and intra-species communication in bacteria and also in bacteria-host signaling. TCS = two component system, AIPs = autoinducing peptides, AI-1 = autoinducer 1, AI–2 = autoinducer, AI-3 = autoinducer 3, AQs = alquil quinolones,DSF = diffusible signal factor, c di-GMP = cyclic di-GMP, AHLs = acyl homoserine lactones.

Please cite this article in press as: A.M. Holban, et al., Control of biofilm-associated infections by signaling molecules and nanoparticles, Int J Pharmaceut (2016), http://dx.doi.org/10.1016/j.ijpharm.2016.02.044