International Journal of Antimicrobial Agents 46 (2015) 494–501

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: http://www.elsevier.com/locate/ijantimicag

Review

Bacteriocins and their position in the next wave of conventional antibiotics

Veronica L. Cavera [a], Timothy D. Arthur [a], Dimitri Kashtanov [b], Michael L. Chikindas [b,*]

[a] Department of Biochemistry and Microbiology, Rutgers State University, 76 Lipman Drive, New Brunswick, NJ 08901, USA
[b] School of Environmental and Biological Sciences, Rutgers State University, 65 Dudley Road, New Brunswick, NJ 08901, USA

ARTICLE INFO

Article history:
Received 23 February 2015
Accepted 15 July 2015

Keywords:
Bacteriocins
Antimicrobial peptides
Antibiotic targets
Synergy
Future medicine

ABSTRACT

Micro-organisms are capable of producing a range of defence mechanisms, including antibiotics, bacteriocins, lytic agents, protein exotoxins, etc. Such mechanisms have been identified in nearly 99% of studied bacteria. The multiplicity and diversity of bacteriocins and the resultant effects of their interactions with targeted bacteria on microbial ecology has been thoroughly studied and remains an area of investigation attracting many researchers. However, the incorporation of bacteriocins into drug delivery systems used in conjunction with, or as potential alternatives to, conventional antibiotics is only a recent, although rapidly expanding, field. The extensive array of bacteriocins positions them as one of the most promising options in the next wave of antibiotics. The goal of this review was to explore bacteriocins as novel antimicrobials, alone and in combination with established antibiotics, and thus position them as a potential tool for addressing the current antibiotic crisis.

1. Introduction

The overwhelming increase in antibiotic resistance is presently recognised as a global crisis and as such requires the immediate attention of the pharmaceutical industry, academia and government institutions [1]. The increasing rate of bacterial resistance and the inability to discern mechanisms of inhibiting them impedes the rate of antibiotic discovery. Antibiotic resistance is not new; it is a phenomenon that has been documented since the discovery of penicillin [2,3]. The current wave of resistance is problematic because the rate at which resistance is occurring is equivalent to the ubiquity of resistant pathogens. For more information on resistance mechanisms, the authors suggest the review by Cotter et al. [4]. Furthermore, there are additional complications of antibiotic overuse such as killing of healthy microbiota and environmental contamination that cause immediate and prolonged ecological issues.

Both novel substances and innovative methods are constantly being evaluated to address the rapid spread and development of drug-resistant infections in nosocomial settings. Although the next step is uncertain, it is clear that a viable alternative is necessary to ensure an efficacious paradigm shift that can stymie the epidemic of resistance. The dimensions of the antibiotic crisis have been discussed in reviews [1,5]. These articles deftly describe the dimensions that have led to this crisis but do not offer substantive information into alternatives.

Antimicrobial peptides have been in the forefront of antibiotic alternative research for decades, but their usefulness has failed to be substantively explored. In particular, bacteriocins, antimicrobial peptides of bacterial origin, are positioned as potentially significant contributors to the paradigm shift owing to the wide variety of commercially available formats. In addition, many groups are actively researching and developing existing and novel bacteriocins and bacteriocin-like substances.

This review aimed to position bacteriocins as possible alternatives to conventional antibiotics or, perhaps, as novel nature-derived stressors that can be used in formulations with synergistically acting antibiotics as complementary agents. The latter approach is in agreement with the strategy proposed by the National Center for Complementary and Integrative Health of the National Institutes of Health (NCCIH NIH).

2. A brief introduction and history of bacteriocins

Bacteriocins were first identified in 1925 and are defined as ribosomally synthesised, proteinaceous substances that inhibit the growth of closely related species through numerous mechanisms [6,7]. Production of these proteins is widespread among bacterial species and it is suggested that virtually all bacterial

* Corresponding author. Tel.: +1 848 932 5405.
E-mail address: tchikindas@aesop.rutgers.edu (M.L. Chikindas).

http://dx.doi.org/10.1016/j.ijantimicag.2015.07.011
0924-8579/

species synthesise bacteriocins [8,9]. Such production is made possible by relatively simple biosynthetic machineries that are often associated with elements such as plasmids and conjugative transposons [10,11]. This process is further simplified by the fact that associated genes are often clustered on plasmids, chromosomes or transposable elements. This ubiquity posits bacteriocins as highly appealing.

There have been multiple classifications for bacteriocins. This controversy has led to such divisions as 'true bacteriocins' such as colicins, and those more recently discovered from *Lactobacillus* spp. [12] and other lactic acid bacteria (LAB). Whilst colicins are group structured [13], bacteriocins from LAB have undergone several classifications from being placed into four groups [14] to more recent groupings. There are even more subclassifications based specifically on the taxonomy of the producer micro-organism, such as those synthesised by enterococci [15]. The classification system used in this review divides bacteriocins by modification and size; bacteriocins of Gram-positive micro-organisms, such as those produced by LAB, identified as class I, undergo post-translational modifications, whilst class II undergo either no or minimal modifications. In addition, bacteriocins >10 kDa are parsed into a third class [12]. Bacteriocins from Gram-negative bacteria are divided [16,17] into small peptides, such as microcins, and large peptides, such as colicins [18–20]. Further subdivisions exist within these broader categories, including instances of homology in motifs [21].

Currently, there is extensive research performed on bacteriocins, especially as the US Food and Drug Administration (FDA) regulates their usage as a food preservative. As of 2012, 62 genera encompassing 195 bacterial species are considered as microbial food cultures with a history of safe use for fermentation purposes [22]. Given this considerable endeavour, various targets and efficacies have been determined, further strengthening their position in the next wave of therapies.

3. Bacteriocins utilise some of the conventional drug targets

Current methods of identifying novel antibiotics generally fall into one of two categories, synthetic chemical efforts or isolation of new natural products. Examples of more recent synthetic chemical efforts include high-throughput screening of chemical libraries and targeted structure-guided experiments [23,24]. In addition, there are groups devoted to isolating and screening various natural and nature-derived sources.

Conventional antibiotics fall into five major categories with respect to their targets. These targets include: (i) bacterial peptidoglycan/cell wall disruption; (ii) protein biosynthesis; (iii) folate biosynthesis; (iv) DNA replication and transcription; and (v) disruption of the bacterial membrane [17,25–30].

These are considered the major clinically validated antibacterial targets. Bacteriocins are capable of inhibition of four of these pathways as well as some novel pathways. To illustrate the versatility of bacteriocin targets, we have expanded the conventional list of targets. Fig. 1 displays targets of both antibiotics and bacteriocins, their general location, and examples of each capable of inhibition of these targets.

Bacteriocins can inhibit closely related bacterial species, spores and have even shown instances of fungicidal activity [9,31]. In comparison, antibiotics have been generally regarded as more broad-spectrum with numerous side effects [1]. The side effects and increased incidence of bacteriocin resistance are two topics that require further research. Bacteriocins are effective against four of the aforementioned clinically relevant antibiotic targets. Some bacteriocins have been studied in vivo and were successful in inhibiting the targeted pathogens (for review see [27]). These findings support the necessity for further clinical investigation.

3.1. Inhibition of cell wall biosynthesis

Antibiotics that target cell wall biosynthesis include those in the β-lactam group. A recent decrease in β-lactam efficacy against common nosocomial infections has prompted the need for viable alternatives. Pathogens have developed β-lactam-degrading enzymes such as carbapenemases and penicillinases [32]. In light of this trend, there is great potential for the integration of bacteriocins, specifically lantibiotics, into new therapies. Despite this potential, the prevalence of lantibiotic resistance can increase when organisms are exposed to subinhibitory levels of lantibiotics for extended periods of time [33,34]. The cell wall is widely regarded as an excellent target for the development of novel technologies as its synthesis is highly conserved across pathogens and is absent from mammalian cells [34,35]. Furthermore, the cell wall is critical to overall bacterial survival in that it regulates cellular integrity and morphology, particularly in cases of internal osmotic pressure fluctuations. Therefore, prevention of cell wall biosynthesis is a critical target [35]. Current studies in *Escherichia coli* and *Bacillus subtilis* have indicated MreB, a bacterial actin homologue, as critical for maintenance of shape, and penicillin-binding proteins (PBPs) as enzymatic regulators. MreB rotates for maximum uniform distribution of peptidoglycan insertion sites, and subsequent motion is dependent on the availability of these subunits [36]. PBPs, particularly PBP2, are responsible for covalent cross-linking of glycan strands during growth [37]. Current antibiotics target cell wall synthesis at four different stages of peptidoglycan development: (i) inhibition of the synthesis of lipid II; (ii) inhibition of the undecaprenyl carrier lipid; (iii) binding of lipid II; and (iv) binding and blocking of the active sites of PBPs [3]. Some examples of antibiotics that target these sites include penicillins, glycopeptides, carbapenems, monobactams and cephalosporins [30]. The coupling of MreB motion and PBP2 regulation appears highly conserved among bacterial species [36–38]. Further analysis of how bacteriocins can affect MreB and/or PBP2 could prove extremely beneficial given this high level of conservation.

Nisin A, produced by *Lactococcus lactis*, one of the most frequently referenced bacteriocins, possesses multiple modes of action. This lantibiotic docks to lipid II, a membrane-bound precursor of the cell wall, and inhibits cell well synthesis. In addition, following lipid II docking, pore formation by nisin molecules arranged as pore-forming 'units' can be induced, which rapidly kills cells. At high quantities, this process can be divided into two stages, with the first being bacteriostatic and the second bactericidal [3,33,38]. Nisin has also been found to act as a lytic agent [39]. Nisin is known to effectively inhibit numerous Gram-positive bacteria, leading to its usage in the food industry [40–42]. Similarly, nukacin ISK-1, produced by *Staphylococcus warneri*, inhibits cell wall synthesis by binding lipid II but it has not been shown to induce pore formation [43–45]. This bacteriocin has been shown to be potent for the treatment of meticillin-resistant *Staphylococcus aureus* (MRSA) biofilms [43]. It has been found that ring A is responsible for binding lipid II [45]. *Microbispora* sp. strain ATCC-PTA-5024 produces the lantibiotic NAI-107, which also binds to lipid II leading to inhibition of vancomycin-resistant enterococci and MRSA [46,47]. Other lantibiotics of interest include lacticin 481, a tricyclic lantibiotic that contains a lipid II-binding motif but inhibits PBP1b-catalysed peptidoglycan formation [48,49].

The aforementioned bacteriocins show great promise in preventing cell wall biosynthesis by binding lipid II. Future research may be directed towards reconstruction studies in which there is a better understanding of the molecular mechanism of development and the linkage of MreB, PBPs and lipid II. Understanding how these interact could potentially indicate novel antimicrobial targets.