Biochimica et Biophysica Acta 1858 (2016) 926–935

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Using adjuvants and environmental factors to modulate the activity of antimicrobial peptides☆

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ARTICLE INFO

Article history:
Received 16 October 2015
Received in revised form 22 December 2015
Accepted 29 December 2015
Available online 2 January 2016

Keywords:
Antimicrobial peptides
Environmental factors
Adjuvants
Antibiotic targets
Resistance mechanisms
Synergy

ABSTRACT

The increase in antibiotic resistant and multi-drug resistant bacterial infections has serious implications for the future of health care. The difficulty in finding both new microbial targets and new drugs against existing targets adds to the concern. The use of combination and adjuvant therapies are potential strategies to counter this threat. Antimicrobial peptides (AMPs) are a promising class of antibiotics (ABs), particularly for topical and surface applications. Efforts have been directed toward a number of strategies, including the use of conventional ABs combined with AMPs, and the use of potentiating agents to increase the performance of AMPs. This review focuses on combination strategies such as adjuvants and the manipulation of environmental variables to improve the efficacy of AMPs as potential therapeutic agents. This article is part of a Special Issue entitled: Antimicrobial peptides edited by Karl Lohner and Kai Hilpert.

1. Introduction

The discovery and subsequent development of antibiotics have arguably been among the most important medical advances in history [1–3]. The precarious nature of existence without access to modern antibiotics is illustrated by 2012 World Health Organization data from sub-Saharan Africa, where more than 2 million deaths (>20% of total deaths) were attributed to a variety of infectious diseases, excluding HIV and malaria, which together accounted for an additional 2 million deaths [4]. The introduction of sulfa-drugs (sulfonamides) and penicillin (β-lactams) in the early decades of the 20th century led to the golden age of antibiotics in the 1950s and 1960s during which time many different classes of antibiotics with a variety of different microbial targets were developed [5,6].

Successful microbial targets (Fig. 1) have included a variety of enzymatic steps in the synthesis of peptidoglycan (PepG) of the bacterial cell wall, different sites involved in ribosomal protein synthesis, enzymes of folic acid metabolism, DNA synthesis (DNA gyrase), RNA synthesis (RNA polymerase) and the microbial plasma membrane [2,3,7,8]. The pattern of antibiotic development over the last 50 years, however, is a cause for concern. Most of the new ABs introduced since 1962 have simply been modifications of existing ABs resulting in 2nd and 3rd generation drugs against the same targets [2,3,5,7,9]. Even the two most recent drug classes, oxazolidinones such as linezolid (2000) and lipopeptides such as daptomycin (2003), which inhibit new bacterial targets, were actually discovered nearly 20 years earlier [2]. Furthermore, there has been a dearth of new broad spectrum ABs over the last 40 years, with a particular need for new methods for treating Gram negative infections as most of the ABs marketed since 2000 have improved activity against only Gram positive organisms [6,7,9]. The development of new ABs has also been severely hindered by an increasingly stringent regulatory environment including the possibly shortsighted requirement that new drugs be as good or better (FDA noninferiority) as existing treatments rather than simply effective, although recent changes such as the REMS program and 21st Century Cures Act have attempted to address some of these issues [2,7,10,11]. Given that treatments for cancer, microbial infections, and HIV, for example, all have problems with multidrug resistance, it seems unwise to limit the number of available drug choices. Fewer options may exacerbate the difficulties in treating resistant organisms and may also limit competition and lead to higher drug prices. Another important consideration is that, by their very nature, AB treatments for episodic infections suffer in competition with the more lucrative development of drugs for chronic conditions such as diabetes, arthritis, and heart disease [1,6,7,10,12].

In order to increase the number and diversity of antimicrobial targets, a variety of new types of screens, including the use of modern genomic methods have been employed, but with mixed success [6,10,13–15]. Although a number of potential new targets have been identified such as peptide deformylase and enzymes of the Type II fatty acid synthesis pathway, most new leads continue to be natural products

☆ This article is part of a Special Issue entitled: Antimicrobial peptides edited by Karl Lohner and Kai Hilpert.

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http://dx.doi.org/10.1016/j.bbamem.2015.12.034
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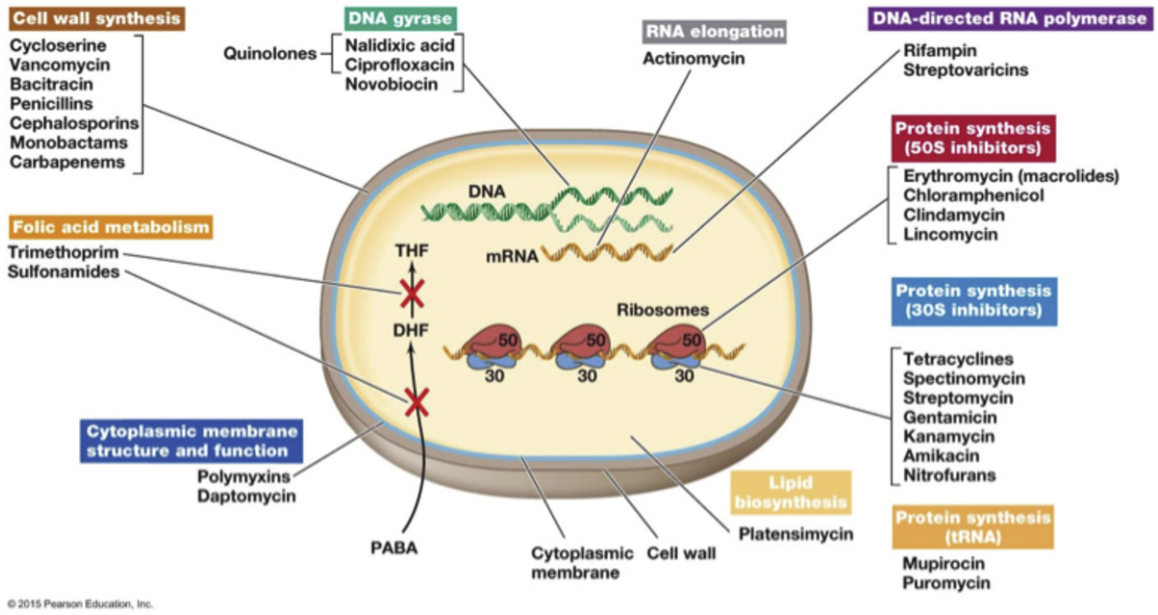

Fig. 1. Targets of antibiotics. Ten different categories of antibiotic targets are depicted. The most effective antibiotics target cell wall synthesis, DNA gyrase, ribosomal protein synthesis (30S and 50S subunits), and folic acid metabolism.
The figure is reproduced, with permission, from Ref. [158].

with the recent discovery of teixobactin the result of a new method designed to allow testing of uncultivated soil bacteria to identify potential new antibiotics [13,16–18].

The paucity of new antimicrobial targets is problematic due to the increasingly formidable problem of microbial resistance to ABs. In recent years, an increase in the incidence of antibiotic resistant and multidrug resistant infections has threatened to reverse many of the gains of the antibiotic era. In 2013, the Centers for Disease Control (CDC) reported that more than 2 million infections and 23,000 deaths are attributed to antibiotic resistant organisms annually in the USA [7,19]. Microbial resistance is not a new occurrence, however. Resistance has been observed for nearly every antibiotic, often occurring fairly quickly after introduction of the antibiotic. For example, resistance against penicillin [8], sulfonamide, streptomycin [3], and more recently daptomycin [9] were all observed shortly after their introduction and in the case of penicillin, before its widespread therapeutic use [17]. In retrospect, this is not surprising as many ABs are naturally occurring molecules produced by soil microbes in an ancient competition between ABs and resistance mechanisms that has been ongoing for billions of years [8,12,20,21].

The naturally occurring AMPs present an interesting case. AMPs have evolved in species as diverse as bacteria to plants to humans and yet remain effective against most microbes [22]. AMPs are part of the innate immune system in both vertebrates and invertebrates and act through a binding event in which the positively charged peptides interact with the anionic plasma membranes of susceptible microorganisms [23,24]. Although the synthesis of the cell wall components of peptidoglycan has been a common target for many ABs, the microbial plasma membrane and other cell surface targets such as the lipopolysaccharide outer membrane (LPS) and lipid A components of Gram negative organisms, and the teichoic acids (TA) of Gram positive organisms have been underexploited targets for commercial ABs [13,25]. Although AMPs primarily target the plasma membrane [24,26, see also ***this issue], it has been observed [27], that pH dependent interactions with other charged molecules of the microbial cell wall or lipopolysaccharide layers [26,28,29] are also important. Following binding of the plasma membrane, the amphipathic AMPs then interact with the nonpolar membrane lipids [30] and cause cell death due to membrane disruption, although other mechanisms have also been implicated [31].

AMPs have several important advantages in addition to their low levels of natural resistance. AMPs sterilize rapidly at micromolar concentration, are able to target quiescent cells, and often have very broad spectrum activity. Disadvantages of AMPs include all of those common to peptide drugs: (a) they are subject to proteolysis in the digestive tract, thus limiting oral administration, (b) peptide drugs tend to be expensive, and (c) intravenous and subcutaneous injections are also limited due to the possibility of both proteolysis and immunogenicity [22]. A significant disadvantage, unique to AMPs, is that in addition to targeting microbial membranes, AMPs also target red blood cell and other host membranes, although typically at higher concentrations. The resulting cytolysis is an impediment to the development of AMPs as systemic drugs and efforts are underway by a number of groups to increase the therapeutic index (cell selectivity) for this class of drugs [32–36].

Though limited in common routes of delivery, AMPs are well suited for topical and other surface applications [37–39]. In fact, most clinical trials to date involve the treatment of diabetic ulcers and other skin infections such as thrush, as well as infections related to cystic fibrosis [40], while additional applications such as in treatments for eye infections and as additives to cosmetics are under investigation [22,39,41]. A potential advantage of topical applications highlighted in this review is that one can adjust environmental factors such as pH, ionic strength, and the concentration of specific ions, and make use of adjuvants to potentiate the activity of the AMPs. Thus, a comprehensive understanding of the effect of environmental factors and adjuvants is needed to further the development and application of AMPs as successful drug therapies.

2. Resistance mechanisms

Multidrug resistant bacteria are widespread and are encountered increasingly often in infections [2,3,5]. Bacteria have developed a number of common methods of acquiring resistance (Fig. 2). Among these are: (a) enzymatic modification of ABs to inactivate them, (b) mutation of AB target to prevent binding of ABs, (c) overexpression of target molecules, (d) use of an alternate pathway to bypass the action of the AB, (e) efflux pumps in the plasma membrane to prevent buildup of ABs within cell, (f) mechanisms to decrease permeability or entry of ABs,