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Antimicrobial peptides: new drugs for bad bugs?

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Abstract

Antibiotics have been among the most successful classes of therapeutics and have enabled many of modern medicine's greatest advances. However, antibiotic-resistant bacteria are emerging as critical public health threats, with recent accounts of bacterial strains resistant to all approved antibiotics. Antimicrobial peptides (AMPs) are naturally occurring molecules with the potential to serve as the basis for a new class of anti-infectives targeting these difficult-to-treat bacteria. The unique activities and features of AMPs are discussed, with a focus toward the clinical importance of priming the antibiotic pipeline and the role AMPs can fulfill in the future of fighting drug-resistant bacteria.

Keywords

antibiotics; antimicrobial peptides; drug-resistant bacteria; peptide design

Antibiotics, one of the most important medical developments of the twentieth century, are losing effectiveness each year as diverse bacteria become resistant to standard and last resort commercial drugs. While the traditional focus of antibiotic discovery programs in large pharmaceutical companies (and elsewhere) has been centered on small molecule therapeutics, a newer class of molecules has been proposed as a potential source of novel anti-infectives [1]. Antimicrobial peptides (AMPs), also called host defense peptides, are a diverse class of molecules that function as a first line of defense against microbial threats. More than 2000 AMPs have been discovered and isolated from organisms as diverse as

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plants, insects, amphibians, humans and even bacteria [2]. Considerable effort continues to be directed toward the identification, isolation and activity testing of new AMPs.

AMPs can, in general, be divided into four categories based on their predominant secondary structure: i) α-helical; ii) β-sheet; iii) mixed α-helix/β-sheet and iv) extended [3,4]. Many prototypical AMPs have a net positive charge that mediates their selective activity against bacterial cells that carry a net negative charge [1]. AMPs are also generally amphipathic, which is the result of a physical segregation of the charged/polar and hydrophobic residues to opposite 'sides' of the molecule in the active structure. While the antimicrobial activity of AMPs was initially proposed to occur by membrane disruption (as a general result of three proposed models: the barrel-stave, carpet or toroidal pore models [5], it has become increasingly clear that AMPs can also act through mechanisms involving interaction with membrane-associated protein targets or by penetration into the bacterial cytoplasm and interacting with intracellular targets [6,7]. In addition to these direct antimicrobial effects, AMPs recently have been demonstrated to function in host immune modulation, often by enhancing protective immunity and suppressing inflammation [8].

While intensive AMP research has led to increased understanding of the mechanism of action (MOA) and the breadth of antimicrobial activity, the threat of antibiotic-resistant bacteria continues to increase. Therefore, a relevant question is, 'Are we finally ready for clinical use of AMPs?' Below we provide our perspective for the future of AMPs as clinical antibiotics.

Expert opinion

Refilling the antibiotic pipeline is one of our most pressing medical needs. Over the past few decades, antibiotic research and development has steadily declined to the point where only two new antibiotics have been approved for general use since 2008. Among the large pharmaceutical companies, only four have active antibiotic discovery programs, down from 18 in 1990 [9]. The last truly new class of antibiotics was introduced in 2003 (daptomycin, a lipopeptide). It has been > 40 years since the introduction of fluoroquinolones, the last new class of antibiotics that treat infections caused by Gram-negative bacilli. Multidrug-resistant (MDR), extensively drug-resistant (XDR) and pandrug-resistant (PDR) organisms such as *Klebsiella* and *Acinetobacter* species are becoming increasingly prevalent and the death rate from these infections can approach 50%. Given these challenges, we believe that it is a critical time for an expanded examination of the clinical potential of AMPs and suggest that AMP-based therapeutics be more seriously considered as a means to treat these new, and increasingly deadly, bacterial threats.

AMPs have only been tested in clinical trials relatively recently, and to date, none have received US Food and Drug Administration (FDA) approval, with the exception of gramicidin for topical administrations. Magainin Pharmaceuticals provided early high hopes for the field, with impressive data in early Phase I and II clinical trials using the compound pexiganan (a synthetic analog of the AMP magainin) to treat diabetic foot ulcers. Ultimately, however, the compound was not approved by the FDA because it did not provide superior performance when compared to traditional antibiotics used in treating foot ulcers. This early

setback with pexiganan combined with the difficulty and expense associated at that time with manufacturing peptides markedly suppressed enthusiasm for AMP-based therapeutics development. While there are currently no marketed drugs based on AMPs (with the same exception as above), the present state of bacterial antibiotic resistance, combined with recent scientific advances in the field and progress in the synthesis, functional design, and manufacture of peptides, has increased the interest in commercialization of antibiotics based on AMPs [10]. Currently, there are only a small number of companies researching AMPs as therapeutics, but there are at least 10 AMP-derived compounds in varying stages of clinical development [10].

As commercialization interest in AMPs increases, it is important to consider that the majority of AMPs currently in clinical trials are analogs of natural AMP sequences or modified derivatives thereof. Natural AMPs, by virtue of their diverse origins and evolution, target many microbial species and can exhibit potent *in vitro* activity. However, low *in vivo* activity, the labile nature of peptides and potential toxicity concerns, which have prevented development of systemic applications, have hindered AMP clinical development.

In an attempt to address the clinical concerns associated with many natural peptides, a new approach to AMP research and discovery has emerged in recent years. In contrast to isolating and/or modifying natural AMPs for use as therapeutics, this new approach calls for the design of synthetic sequences, which are not known or expected to exist in nature and that are the result of optimizing sequence and chemical characteristics that are common to many types of AMPs. To this end, a number of groups have used *de novo* designed peptide sequences in an effort to overcome some of the limitations observed with natural sequences, such as decreased activity in serum and/or blood and systemic toxicity [11–14]. Success with designed AMPs *in vivo* [15,16] and recent *in vitro* activity data against MDR, XDR and PDR clinical isolates of *Klebsiella pneumoniae* and *Acinetobacter baumannii* highlight the advantages and the potential of rationally designed AMPs [17].

AMPs provide the potential for not only a new class of antibiotic but also the introduction of a new MOA into the antibacterial arsenal. While the exact MOA of diverse AMPs may differ, it is clear that AMPs can have complex, multi-target mechanisms that can be distinct from those of approved antibiotics, which may confound the generation of resistance development [8]. Additionally, since resistance to traditional antibiotics does not appear to confer resistance to AMPs [18], development of therapeutics based on AMPs has the added benefit of immediately addressing the bacterial infections causing the greatest unmet medical need.

In addition to a unique MOA and activity against the most highly resistant organisms, AMPs are an important class of molecules because of additional bioactivity features that add value beyond what has been achieved with traditional small molecule antibiotics. One perhaps surprising feature is the potent AMP activity that has been demonstrated against bacterial biofilms [10,19], which are structured ‘communities’ of bacteria that are established during many types of infections and that can be refractory to treatment with traditional antibiotics, thus complicating and extending treatment. It is important to note that AMPs tested to date have demonstrated either good antibiotic activity or good anti-biofilm activity but not

necessarily both [20]. As AMPs have been demonstrated to prevent biofilm formation, and more importantly to disrupt established biofilms, they could become an important tool in fighting these difficult-to-treat infections. A second feature that is unique among antibiotics is that AMPs can display a wide range of antiviral properties [5,21]. Many serious infections, particularly pneumonia, can progress to a mixed viral and bacterial infection, with each pathogen exacerbating the effects of the other. While these results are from *in vitro* studies, AMPs could be the first potential therapy with the ability to treat both viral and bacterial infections with one treatment. This unique spectrum of antibacterial and antiviral activity also makes AMPs interesting potential prophylactics for use in the event of a general exposure to an airborne bioterror attack with unknown or mixed agents, particularly among soldiers on the battlefield. In the event of an exposure, an individual could use an AMP-containing inhaler to reduce the infectious dose to subclinical or sublethal levels. These potential uses add a level of value to the concept of AMPs as next-generation anti-infectives that is not currently attained by conventional antibiotics.

There is now a possibility that future generations will inherit a medical system that with respect to infectious diseases more closely resembles that of the 1930s than the present day. Antibiotics are, in many respects, the pillars of modern medicine and have made many of our greatest medical achievements possible by allowing aggressive action without fear of infection. Transplant medicine owes much of its success to antibiotics, as they are used prophylactically to prevent infection in chronically immunosuppressed organ recipients. Likewise, many cancer therapies are possible only because the use of antibiotics prevents infections that would occur otherwise. Novel antibiotic discovery is challenging, as recent studies have demonstrated that targets unique to bacteria compared to mammalian cells may have already been exhausted [22]. The prevalence of natural AMPs in diverse species clearly indicates the ability of these peptides to inactivate diverse bacterial species in various bioenvironments. Thus, future studies on AMPs should be focused on elucidating the critical structural determinants and mechanisms of activity of natural AMPs and the application of these structure–function relationships to the rational design of synthetic AMPs to optimize antimicrobial activity and to minimize toxicity and production costs. In this regard, AMPs may represent the next ‘penicillin’ paradigm as a natural model for the future development of novel antibiotics.

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