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Conjugates and nano-delivery of antimicrobial peptides for enhancing therapeutic activity

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ABSTRACT

The current global crisis of antibiotic drug resistance is driving the search for novel treatment approaches. Antimicrobial peptides (AMPs) are small molecular weight proteins with varying number of amino acids found in both eukaryotes and prokaryotes. They have recently been targeted as novel antimicrobial agents with the potential to treat multiple-drug resistant infections. Their conjugation with various classes of materials such as antibiotics, polymers, DNA, salts, phenolic derivatives and their delivery via nano carrier systems are strategies being used to enhance their therapeutic efficacy. An update and understanding of their applicability as conjugates and nano delivery are essential to optimise their development and activity. This review focuses on computational studies depicting their permeation through model membranes and identification of physicochemical descriptors for activity. It also highlights the potential of AMPs and their conjugates and encapsulation into nano delivery systems for improving activity. Further, research to realise their potential as conjugates and delivery via nano carrier systems are also identified. To our knowledge, this current review presents the first account that comprehensively highlights AMPs targeting various microorganisms, and their conjugation to different compounds to showcase the potential for nano delivery alone or in their respective conjugates for enhanced activity.

1. Introduction

Infectious diseases are one of the leading cause of mortality globally, despite modern technological advances of the 21st century on new drugs and diagnostic equipment used to improve healthcare [1,2]. Over the past 10 years in particular, re-emerging infectious diseases have challenged researchers and the public health systems in their efforts to curb the rise of pathogenicity [3–12]. Bacteria possess numerous drug target sites, with the number of exploited sites being relatively small [13]. This gap in the exploitation of bacterial intracellular targets allows for the synthesis and design of newer antimicrobial agents. Antimicrobial drugs have various modes of action, and depend on factors such as their structural conformation and affinity to certain target sites [14]. The most effective antibiotics act as inhibitors of cell wall synthesis (e.g. penicillins, cephalosporins, bacitracin and vancomycin) [15], cell membrane function (e.g. polymixin B and colistin) [16], protein synthesis (e.g. aminoglycosides, macrolides, lincosamides, streptogramins, chloramphenicol, tetracyclines), nucleic acid synthesis (e.g. quinolones, metronidazole, and rifampin) and other metabolic processes (e.g. sulfonamides and trimethoprim) [17]. Despite the development of numerous potent antibiotics, infections continue to be a challenge to treat, with the bacteria developing strategies to circumvent their action [18–21].

While antibiotics have revolutionised the therapy of infections, several disadvantages with current dosage forms have been observed. These include inadequate concentration at target infection sites, poor penetration of the antibiotics, side effects and poor adherence [22–24]. These limitations have contributed to antibiotic resistance by microorganisms, causing infections on a global scale [25]. The World health organization (WHO) also identified other causes of drug resistance that include the inappropriate use of antibiotics, lack of quality medicines, animal husbandry practices, poor infection control, weak surveillance systems and a lack of progress in developing new vaccines to combat drug resistance [26]. The reduction in effectiveness of a drug [27] is mainly used in the context of pathogenesis, and occurs through a number of mechanisms, such as: (a) drug modifications by enzymes, such as β-lactamases, (b) target site alterations, (c) metabolic pathways alterations, and (d) reduced drug accumulation due to efflux pump

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Table 1
Examples of bacteria resistant to antibiotics.

Bacteria	Type	Drugs resistant to	Ref
Methicillin-resistant *Staphylococcus aureus* (MRSA)	Gram (+) cocci	Vancomycin, Linezolid, Daptomycin, Eicoplanin	[214]
Vancomycin resistant *Staphylococcus aureus* (VRSA)	Gram (+) cocci	Erythromycin, Vancomycin	[215]
S. pneumoniae	Gram (+) diplo-coccus	Doxycycline, Erythromycin, Penicillin G	[216]
E. faecium, VRE	Gram (+) cocci	Vancomycin, Streptomycin, Gentamicin, Penicillin, Ampicillin	[217]
E. coli	Gram (−) rods	Ciprofloxacin, Levofloxacin	[218]
P. aeruginosa	Gram (−) rod	Imipenem, Meropenem, non-antipseudo-monal Penicillins	[219]
K.pneumoniae	Gram (−) rods	Colistin,	[220]
A. baumanii	Gram (−) rod	Imipenem, Meropenem	[221]

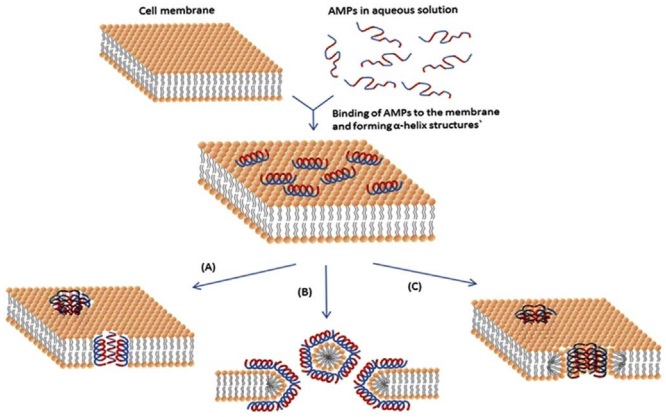

Fig. 1. The various AMPs mechanisms. A represents the Barrel-Stave model (AMPs penetrate the membrane in a perpendicular fashion). B represents the carpet Model (sections of the membrane are coated with AMPs). C represents the Toroidal-pore model (AMPs are in a constant interaction with the membrane phospholipid head groups). AMP Hydrophobic and hydrophilic parts are represented by the colour blue and red respectively. Permission granted [69]. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

activity [28,29]. Drug resistance has led to the inadequacy of current dosage forms and has significantly hindered the efficacy of antibiotics [30]. This includes resistance to bacteria, such as Methicillin-resistant *Staphylococcus aureus* (MRSA) (resistant to beta-lactams), *E. faecium* (resistant to streptomycin), *K. pneumonia* (resistant to 2nd and 3rd generation cephalosporins) and *A. baumanii* (Table 1) [31]. The proliferation of multidrug-resistant strains has led to the search for effective therapeutic agents, and has ignited research into the design and synthesis of novel antimicrobial molecules [32,33]. The development of alternative therapeutic agents remains one of the major challenges to circumventing the problem of drug resistance [34]. Antimicrobial peptides (AMPs) represent a new class of potential drug candidate and are proteins of smaller molecular weight (2–8-kDa) that are broad spectrum in their activity against pathogenic bacteria, viruses and fungi [35,36]. They are also known as host defence proteins (HDPs), and are part of the innate immune system found in all classes of life [37]. The discovery of AMPs dates back to 1939, with gramicidins being discovered first and isolated from *B. brevis* [38]. Gramicidins have been used to treat infected wounds on the skin of guinea-pigs [39], which led to their consideration for clinical use, after which they were commercially synthesized as antibiotics. The number of AMPs discovered and/or synthesized to date is above 5000 [40].

AMPs are either natural based obtained from prokaryotes and eukaryotes [41], or synthetic based. They are divided into four structural groups' viz. (a) β-sheet; (b) α-helical; (c) loop and (d) extended peptides with broad spectrum activity [42], with α-helix and β-sheets specifically being more common [43]. As AMPs are constructed by coupling amino acids, it is easy to modify their structure [44], which is an advantage in designing various combinations. This ability also allows for the possibility to change the AMP targets and improve their stability against the degradative effects of proteases [45]. AMP activity occurs mainly by disrupting the integrity of the membrane protein, inhibiting DNA and RNA synthesis, or disrupting intracellular targets [46]. AMP action is dependent on their cationic charge, which allows them to be attracted to the anionic membrane of its targets and leads to the destruction of the cell membrane [47,48]. Fig. 1 shows the different mechanisms AMPs used to traverse the bacterial membrane [49]. The AMPs membrane penetrating ability is a major advantage over conventional antibiotics, which may find it difficult to cross bacterial cell membrane and make their way into intracellular targets [50]. Several review papers have highlighted the applicability of AMPs as antibacterial agents for enhancing activity against various organisms, such as LL-37, melittin and magainin-II, which are active against *P. aeruginosa, L. monocytogens*, and MRSA respectively [51–53]. A number of AMPs, such as pexiganan acetate, Omiganan, IMX924, Arenicin, Semaglutide and Dulaglutide, were found to be active against Gram positive and negative bacteria, and are now in clinical trials (Table 2). Their intended use was for diabetic ulcers, catheter infection prevention and Type 2 diabetes mellitus [54,55]. Despite their beneficial potent antibiotic activity, their inherent drawbacks, including poor physicochemical stability, a short circulating plasma half-life and a high haemolytic effect [56] have the potential to render AMPs un-usable [57–59]. Various strategies are therefore being used to overcome these limitations. To potentiate their activity, AMPs are also being increasingly explored for conjugation to several classes of materials. The conjugation strategy of AMPs to other compounds amplifies their potential to overcome the current drug resistance crisis [60] as it offers in combination multiple benefits as opposed to the AMP alone. These AMP conjugates can lead to multiple mechanisms of action against bacteria, facilitate self-assembly of AMPs into nanostructures for delivery, achieve intracellular targeting and prolong circulation life [61–64]. Administration of AMPs or its conjugates will eventually require its incorporation into a dosage form for patient administration. The use of current conventional dosage forms will limit the potential of AMPs as they lead to inadequate delivery to the infection site, may not offer protection against degradation by proteases and other degradative enzymes [23,65]. Although it has been noted that the mechanisms of bacterial resistance to AMPs are still not well understood, and their occurrence very unlikely, physico-chemical modifications in the bacterial cell membrane seems to be the first step to developing resistance [66]. Once the bacteria changes the AMP target to make it less susceptible to the action of AMPs, fluidity and bacterial cell permeability decrease due to alterations in the architecture of the outer and inner membranes. Bacterial membrane surface modifications, which can lead to reduced levels of specific membrane proteins and ions, as well as changes in the membrane lipid composition, can promote resistance, which alters the activity of the AMP at its site of action [67,68]. To circumvent this occurrence, the encapsulation or association of these AMPs into nanosized carriers as delivery systems is being explored to achieve targeted delivery to the infection site and reduce resistance [69]. This would provide an added advantage since these nano carriers provide adequate delivery with selective targeting to the infection site as well protection from enzymatic degradation. Also the nano carriers will provide high stability, high carrier capacity, feasibility of incorporation of both hydrophilic and hydrophobic substances, and