

Review

Host Defense Peptides as Effector Molecules of the Innate Immune Response: A Sledgehammer for Drug Resistance?

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Received: 3 July 2009; in revised form: 17 August 2009 / Accepted: 9 September 2009 /

Published: 9 September 2009

Abstract: Host defense peptides can modulate the innate immune response and boost infection-resolving immunity, while dampening potentially harmful pro-inflammatory (septic) responses. Both antimicrobial and/or immunomodulatory activities are an integral part of the process of innate immunity, which itself has many of the hallmarks of successful anti-infective therapies, namely rapid action and broad-spectrum antimicrobial activities. This gives these peptides the potential to become an entirely new therapeutic approach against bacterial infections. This review details the role and activities of these peptides, and examines their applicability as development candidates for use against bacterial infections.

Keywords: host defense peptides; sepsis; innate immunity; antimicrobial peptides; bacterial infection; inflammation

1. Introduction

Throughout our history we have placed human health above our concern for the survival of pathogenic micro-organisms. We have sought the elimination of infectious diseases and the destruction of the micro-organisms that cause those diseases. Antibiotics and vaccines were one of the crown jewels of medical progress in the 20th century. At the start of the 21st century, we find ourselves in a peculiar and perplexing situation in that the pharmaceutical pipeline appears to have become less and less productive. Unfortunately, the initial promise of these miracle drugs was soon set back to some degree by the emergence of resistance to these agents among a broad variety of bacterial species and our ability to identify new agents to cope with the resistant bacteria is diminishing. Due to the threat of development of antimicrobial resistance as a result of misuse and/or overuse of antimicrobial agents, health care professionals should exercise caution and discretion when treating such infections with these agents [1].

In the 1940s hospitals in Europe and North America reported that bacterial infections were resistant to penicillin [2]. It took less than one year for resistance to penicillin to develop and interestingly the first description of penicillinase occurred 1 year before penicillin was ever used clinically [2]. Following this the first methicillin-resistant *Staphylococcus aureus* (MRSA) was identified in the 1960s [3]. From 1980-1986, there was a resurgence of infective diseases and mortality from these diseases in the United States of America increased by 64% to levels not seen since the 1940s [4]. The World Health Organization has stated that MRSA infections are now endemic worldwide. The rate of MRSA in Canadian hospitals increased from 0.46 to 5.90 per 1,000 admissions between 1995 and 2004 [5], which constituted an enormous increase in hospitalization and treatment costs, with the total cost per infected MRSA patient averaging \$12,216 [6]. In 2002, the first clinical isolate of vancomycin-resistant *S. aureus* (VRSA) was identified [7]. The struggle to gain the upper hand against infections continues to the present day. Medical advances against infectious disease have been hindered by changes in the patient population. Immunocompromised hosts now constitute a significant proportion of the seriously infected population, including patients that have been immunosuppressed by clinicians to prevent the rejection of transplants and to treat inflammatory diseases.

Over the past 50 years health care professionals and researchers have been fighting the increasing number of drug resistant bacteria, since such pathogens present significant clinical problems in managing infections that were easy treatable years before. Diseases once thought to have been nearly eradicated from the developed world, including tuberculosis, cholera and rheumatic fever, have risen again with renewed ferocity. These infectious diseases are major causes of morbidity and mortality worldwide. Therefore, a new paradigm in the treatment of infectious diseases is needed that is not compromised by the development of increasingly aggressive resistant strains of microbial pathogens and the rapid reduction of therapeutic efficacies of the available antibiotic therapies.

The increasing resistance of bacteria to conventional antibiotics has encouraged strong efforts to develop antimicrobial agents with new mechanisms of action. Perhaps research has now entered the post-antibiotic era, as there are currently major initiatives to develop new therapies, including host defense peptides (HDPs), which combine antimicrobial activity with angiogenic, immunomodulating and anti-inflammatory properties [8-10]. None of the clinically used antibiotics possess these features.

Equally important as their roles in the mediation of innate immune response are the roles that each type plays in recruiting B and T lymphocytes of the adaptive immune system to engage in specific antipathogen response. Their functions include antimicrobial, anti-endotoxic, anti-inflammatory activities and promoting wound healing. This aim of this review is to discuss the suitability of HDPs as potential therapeutic tools to treat infectious disease.

2. Cationic Host Defense Peptides

As an evolutionary co-development to prevent microbial colonization and tissue damage the production of HDPs of various structural classes have been isolated from a wide range of animal, plant, fungal and bacterial species [12]. As they have successfully retained their antimicrobial activity for millions of years, certain HDPs act as natural antibiotics, showing an exceptionally broad spectrum of activity, ranging from Gram-negative and Gram-positive bacteria to fungi and viruses [13-16]. It is conceivable that the relatively rapid development of microbial-resistance mechanisms may have evolved alongside the variety and high number of HDPs. At present, more than 1,220 HDPs are known, including over 940 HDPs in eukaryotic organisms, listed in three databases [17-19].

According to their molecular composition, size, conformational structure, or predominant amino acid structure, HDPs can be divided into three main classes: linear α -helical structures without disulphide bonds (for example, cathelicidins, magainins and cecropins), β -sheet structures stabilized by characteristic disulfide bridges (for example, defensins), with predominance of one or more amino acids, and loop-structured peptides [13,20-22].

These latter two human classes constitute the less well-studied cathelicidins and defensins (Table 1) [23,24]. The α -helical structured hCAP-18, also known as LL-37, was first described in 1995 and is the only investigated antimicrobial peptide member of the cathelicidin family. Cathelicidins are a family of antimicrobial proteins found in most mammalian species. They consist of a highly conserved N-terminal domain, cathelin, and a variable C-terminal peptide, which is proteolytically released upon demand. It is mainly produced by leucocytes, epithelial cells and mucosal cells where it is stored in specific granules [25]. Its cationic C-terminal 37 amino acid domain, LL-37 displays broad antimicrobial activity mediated through direct interaction with and disruption of the microbial cell membrane. The enzyme responsible for cleavage of the proprotein in neutrophils is serine proteinase 3. In skin, the serine proteases kallikrein 5 and 7 were recently reported to mediate alternative processing of hCAP18 generating several novel peptide fragments, suggesting that peptide profiles may differ between tissues and biological conditions. This opens up a potential new area of research since their functional profile may differ. In addition to being antimicrobial, LL-37 is implicated in diverse biological processes, such as angiogenesis, chemotaxis, cytokine production, histamine release and wound healings [25-28,100]. Due to the simple linear structure of hCAP-18/LL-37 some bacteria are already able to inactivate it by producing peptidases and proteases which degrades the peptide, for instance the V8 and aureolysin proteases from *S. aureus* and the outer-membrane protease PgtE from *Salmonella enterica* serovar typhimurium [29,30].

Table 1. Functions of HDPs in combating infections and modulating the immune response.

Host Defense Peptide	Site of Expression	Immunomodulatory Function
<i>Human cathelicidin (cationic, α-helical structure, consist of a highly conserved N-terminal domain, cathelin, and a variable C-terminal peptide) [13, 16, 23, 25, 51, 53, 54, 75, 79, 95, 100]</i>		
hCAP18/LL37	Neutrophils, keratinocytes, epithelial cells of skin and testis, gastrointestinal and respiratory tract, mast cells, monocytes/macrophages, CD4+ cells, myelocytes, wound and blister fluid, cervix vagina, esophagus, mouth, tongue	Broad antimicrobial activity, antiviral and antifungal activity, endotoxin-binding properties, modulation of pro-inflammatory response, chemotactic, influence of cell proliferation and differentiation, promoting wound healing and angiogenesis, induction of gene expression, induction of adaptive immunity
<i>Human α-Defensins (human neutrophil proteins; closely-related to human cathelicidin, Cys-Arg-rich, cationic, disulfide bridges, β-sheet structure) [22, 36-40]</i>		
HNP-1 to -4	Azurophilic granules of neutrophil granulocytes, B-cells, natural killer cells, T-cells	Killing of phagocytosed microorganisms, antimicrobial activity against Gram-positive and gram-negative bacteria, antiviral (HSV, CMV, HIV-1) properties, exotoxin-inactivation, chemotactic for monocytes, T-cells, immature dendritic cells, upregulation of tumor-necrosis factor α (TNF- α) and IL-1, downregulation of complement activation, promotion of DC activation
HD-5 and -6	Paneth cells granules of neutrophils, natural killer cells	Microbicidal activity against <i>Escherichia coli</i> , <i>Staphylococcus aureus</i> , <i>Listeria monocytogenes</i> , <i>Salmonella typhimurium</i> , <i>C. albicans</i> , induction of IL-8

Table 1. Cont.

<i>Human-β-Defensins (closely-related to human cathelicidin, Cys–Arg-rich, cationic, disulfide bridges, β-sheet structure) [13, 34-36, 61, 105]</i>		
hBD-1	CD4+ and CD8+ T-cells, dendritic cells, epithelial cells of skin, respiratory, gastrointestinal and urogenital tract, trachea, uterus, pancreas, thymus, testis, vagina, gingival intestine, conjunctiva, cornea, lacrimal and buccal mucosa, tongue, salivary gland, mammary glands, limb joints, astrocytes, microglia	Broad antimicrobial activity, antiviral and antifungal activity, chemotactic, induction of chemokines and cytokines, recruiting immune cells, induction of adaptive immunity and pro-inflammatory cytokines such as IL-8, -18 and -20, degranulation of mast cells, promotion of phagocytosis, induction of dendritic cell maturation by TLR-4, LPS and LTS binding properties, inhibition of MMP-inhibitors (TIMP-1/-2)
hBD-2	Mast cells, CD4+ and CD8+ T-cells, dendritic cells, skin, oral, pulmonl, gastric epithelia, conjunctiva, cornea, astrocytes	
hBD-3	Monocytes, CD4+ T-cells, oral, respiratory tract, gastrointestinal tract, urinary and skin epithelial cells, uterus, placenta, testis, esophagus, heart, neutrophils, trachea, skeletal muscle, tongue, kidney, liver gastrointestinal tract, oro-pharynx, tonsils, salivary glands	

Defensins are a group of closely-related, Cys–Arg-rich, cationic HDPs comprised of 29 to 45 amino acid residues. The six conserved Cys residues that are a characteristic feature of defensins form three intramolecular disulfide bridges between the NH₂- and COOH-terminal regions of the peptide, creating a cyclic, triple-stranded, amphiphilic β-sheet structure, making up the characteristic “defensin-like” fold and spatially separated hydrophobic and hydrophilic regions. These three intramolecular disulphide bridges stabilize its β-sheet structures and increase resistance to proteolysis, but also reduce the flexibility [31,32], although disulphide bridges are not necessarily essential for the antimicrobial activity of defensins [33]. Defensins can be divided into α- and β-defensins; the disulfide connectivities in α-defensins are Cys1–Cys6, Cys2–Cys4 and Cys3–Cys5 (the number indicates the location of the Cys residue in the amino acid sequence from the N-terminus), while in β-defensin they are Cys1–Cys5, Cys2–Cys4 and Cys3–Cys6 [34].

In human neutrophils, defensins comprise 30–50% of the granule proteins [35,36]. In human skin, defensins are produced mainly by keratinocytes, neutrophils, sebocytes or sweat glands [22] and are either expressed constitutively or after an inflammatory stimulus. α -Defensins secreted by neutrophils can be detected in biological fluids [37–40]. The concentration of α -defensins in human plasma under normal physiological conditions is about 40 ng/mL, as measured by ELISA [37]. This concentration increases 2- to 4-fold in patients with an inflammatory syndrome and reaches micromolar concentrations in septic patients [38]. In the plasma, α - and β - defensins bind unspecifically to high mass plasma proteins such as serum albumin, α 2-macroglobulin and C1 complement, which decreases their anti-viral and anti-tumour activity [39,40]. Both α - and β - defensins have been found in human body fluids during inflammatory lung diseases, urinary tract infection and in tears after ocular surface surgery [22,41–46]. The relative structural stability of defensins has not caused their loss during the course of evolution and it can therefore be proposed that certain activities, such as the interaction with dedicated receptors, might have favored the development of more flexible peptides such as hCAP/LL-37. A better understanding of the function of hCAP/LL-37 and defensins in immunity has implications for the development of potential clinical therapeutics for the treatment of infection or cancer.

Recent studies have investigated the dichotomous role of HDPs in cancer biology. It has been shown that hCAP/LL-37 activates tumor cells resulting in increased cell growth *in vitro* and in an animal model [47,48]. In contrast, Bose *et al.* demonstrated that human beta defensin-1 (hBD1) induces rapid cytolysis of prostate cancer cells and that the PAX2 oncogene suppresses hBD1 expression in prostate cancer [49].

3. Activities of Host Defense Peptides

A microbial pathogen has the potential to enter any part of a host organism. Damaged skin can be a major portal of entry and allow multiplication of pathogens; tetanus and burn wound infections are clear examples. As mentioned previously, the innate immune system is the first line of defense as the adaptive immune response is not rapid enough to control the onset of infections. HDPs play a significant role in this process [14,15,50,51]. Their characteristics, including angiogenesis, anti-microbial, chemotaxis, cytokine production, histamine release, lipopolysaccharide (LPS)-binding properties and other immunomodulatory activities can lead to control infection and allow the appropriate activation of adaptive immune responses [52–54].

A correlation between the severity of the disease and the level of HDP production has been demonstrated in several studies. [55,56] Morrison *et al.* could demonstrate increasing susceptibility to infections caused by *S. aureus* in β -defensin-2 knockout mice and isolated Dermcidin (DCD) peptide DCD-1L produced by eccrine sweat glands in the skin has been shown to stimulate the production of cytokines/chemokines by human keratinocytes [55,56]. Reduced expression of DCD in sweat of patients with atopic dermatitis has been associated with high susceptibility of these patients to skin infections and altered skin colonization [57]. In contrast, overexpression or exogenous application of HDPs have a protective effect in animals [51,58,59]. Clinically overexpression can lead to increased protection against skin infections as seen in patients with psoriasis, rosacea and inflammatory skin-diseases such as acne vulgaris which is rarely associated with clinical superinfection [60]. The

expression of many HDPs increases during infection and inflammation. For instance HBD-2 is upregulated in various cell types such as monocytes, epithelial cells and keratinocytes during bacterial infections and by stimulation from different bacterial components that activate the Toll-like receptor (TLR) to nuclear factor (NF)- κ B pathway [61-63]. In addition, decreased defensin levels in burn injury may facilitate infection and subsequent sepsis [64].

Human skin is always in contact with the environment and is covered with a characteristic microflora of non pathogenic bacteria (commensals). HDPs are mainly associated with inflammatory lesions and wounds, but some are also focally expressed in skin, including β -defensins, RNase 7, S100-protein Psoriasin and hCAP-18/LL-37 in the absence of inflammation [22]. This may be due to an imbalance between impaired defense and increased susceptibility to infection [16].

Hancock *et al.* demonstrated that HDP antimicrobial activity was not necessarily required for protection *in vivo* and an HDP with no antimicrobial activity was found to be protective in animal models of *Staphylococcus aureus* and *Salmonella enterica* infection, implying that a host defense peptide can protect by exerting immunomodulatory properties [54]. HDPs have hitherto been considered for their antibiotic properties, however, some of them, particularly defensins, evolved to also exhort a large variety of other functions [54]. This raises the question as to what HDPs really are: are they principally antimicrobials or are they modulators of innate and adaptive immune responses, or both? It has been proposed that some HDPs, for example defensins, have traded their antibiotic capacities to acquire immunomodulatory functions presumably to reverse suppressive microbial functions and to elicit more robust host inflammatory and adaptive responses [54].

4. Strategies and Functional Properties of Host Defense Peptides

HDPs belong to the most rapid evolving group of mammalian peptides [65,66] and have developed broad-spectrum antimicrobial properties, such as direct killing of Gram-negative and Gram-positive bacteria, fungi, and parasites [12,67]. A therapeutic goal is to engineer peptides with high efficacy and target specificity. In order to achieve this goal, the mechanisms of actions of the HDPs must be understood.

There are distinctive different external and internal target mechanisms. One can distinguish between peptides that permeabilize and/or disrupt the bacterial cell membrane and peptides that translocate through the cell membrane and interact with a cytosolic target [68-70].

The antimicrobial activity of HDPs as membrane-agents, possessing a secondary α -helical peptide structure, depends on the presence of an ionic milieu that is comparable to the conditions found in mammalian body fluids [68-70]. The hydrophilic, cationic part is proposed to initiate electrostatic interaction with the negatively charged components of the membrane of microbes; the hydrophobic portion is supposed to permit the HDPs to insert into and permeate the membrane.

The positive charge provides some degree of selectivity towards negatively charged microbial cell envelopes and cytoplasmic membranes. These molecules tend to exhibit intrinsic specificity for microbial invaders and are relatively much less toxic for the metazoan host's cells. This specificity endows the animal with an "innate" immunity, in contrast to the better studied acquired immunity conferred by the clonal expansion of B and T cells. The possible importance of this system as a check on infection is evident when one considers that most bacteria have generation times of 20-30 minutes

whereas the mounting of a specific immune response, dependent on the growth of mammalian cells, may take days or weeks. Although many cationic peptides demonstrate direct antimicrobial activity against bacteria, fungi, eukaryotic parasites and/or viruses [71-73], it has also been established that many also have a key modulatory role in the innate immune response and present an important link between the innate and adaptive immune responses [74].

These HDPs penetrate the outer membrane of Gram-negative bacteria by self-promoted uptake due to hydrophobic or electrostatic interactions with lipid A or polyanionic surface LPS (LPS) [11,75]. Other mechanisms to kill the bacteria cell are possible due to transmembrane channels, which cause changes in permeability of the negatively charged phospholipid membrane. Other models such as barrel stave, lipid flip-flop and carpet models are currently discussed as potential ways for HDPs to penetrate bacterial membranes [76,77]. Furthermore, DNA and other intracellular targets can be affected, leading to cell destruction [77,78]. Many HDPs have additional properties such as anti-endotoxic and anti-inflammatory properties, promote apoptosis, influence cell differentiation, induce cytokine and chemokine release, stimulate mast cell degranulation, possess chemotactic properties and promote wound healing [79-82].

As participants in a competent immune response to invading bacteria, HDPs act as immune activators by induction of transcription and secretion of cytokines such as IL-8, IL-18, TNF- α , GM-CSF, IL-1 β [56,83,84], and induce histamine release from mast cells [85-87]. Certain bacteria are better at provoking an overwhelming immune response from the cells of the innate immune system, such as monocytes, macrophages, and dendritic cells [80,88], which connect the innate immune system with the adaptive immune system. A hyperinflammatory response exposes the organism to the pathological processes of sepsis and may lead to organ failure and death [89-91]. In this case, HDPs may operate as immune response suppressors to prevent a hyperinflammatory response producing severe tissue damage (self-damage) from an overwhelming immune response [23].

Endotoxin covers more than 90% of the outer monolayer of Gram-negative bacteria. For the microbe, endotoxin works as a protective barrier against antibiotics and for the host immune system it is an effector molecule, which is recognized by and activates the innate immune system. In some cases life-threatening complications occur caused by antibiotics stimulated Gram-negative bacteria endotoxin release [92-94]. The human antimicrobial peptide hCAP18/LL-37 is a multifunctional modulator of innate immune responses, its ability to neutralize endotoxin inhibits the production of proinflammatory cytokines such as TNF- α and IL-6 [23,25]. Scott *et al.* demonstrated that structurally different cationic antimicrobial peptides block the interaction between LPS and LPS-binding protein, inhibiting transport and binding of LPS to CD14 receptor [95]. Cirioni *et al.* reported protective effects of hCAP18/LL-37 in lethal sepsis caused by Gram negative bacteria in experimental rat models of peritoneal sepsis. The human cathelicidin LL-37 proved to be similarly successful as three commonly used antibiotics in lowering lethality [96]. Furthermore, HDPs interact directly with eukaryotic cells [43] and can induce alterations in the transcription of hundreds of genes in cells of the innate immune system [97]. In addition cathelicidins and defensins promote cell proliferation and wound healing [98-102].

5. Current Limitations of Host Defense Peptides

As HDPs have a different mode of action compared to conventional antibiotics, they are less affected by multiresistant microbes. However certain HDPs have limitations and some bacteria have developed mechanisms to avoid direct killing by HDPs [103,104], through extracellular and intracellular resistance mechanisms, including reduction of negative surface charge of the cell wall [103,104] and efflux mechanisms [103]. HDPs with a simple linear or α -helical structure are susceptible to proteolysis and are targeted by several microbial proteases. Nevertheless, HDPs can be prevented from reaching the bacterial cell membrane by bacterial secreted proteins. Examples of bacterial secreted proteins include *Streptococcus pyogenes* with streptococcal inhibitors of complement (SIC) proteins, and *Staphylococcus aureus* with staphylokinase [105,106]. In addition, certain HDPs can be actively extruded from bacterial cells, for example by the MtrCDE multiple drug resistance exporter in both *Neisseria gonorrhoeae* and *Neisseria meningitidis* [107,108]. A potential concern that has been noted is that bacteria can also reduce the net anionic charge of their cell envelope, reducing the affinity of HDPs to them. However, these mechanisms are relatively inefficient compared to those that inhibit the effect of conventional antibiotics [109]. Furthermore, only direct killing activity is affected by resistance mechanisms of bacteria whereas immunomodulatory function is untouched.

A new paradigm for the treatment of microbial disease is through the modulation of innate immune responses. The importance of HDPs as antimicrobial agents versus their role as immunomodulators is somewhat controversial. In addition, the pretence of host defense is relative, as the cytotoxic activities of HDPs may have originally evolved to defend the producer bacteria from eukaryotic cell predation [110]. HDPs can trigger autoimmunity through immunomodulation. Autoimmunity and host antimicrobial immunity are inextricably linked, as effector responses that cause inflammatory tissue damage are the same ones that mediate effective host defence. Therefore, immunotherapeutic regimens that target common pathways of the immune system inevitably elicit both desirable and undesirable consequences. HDPs can also interact with human membranes and this is at least partly responsible for cytotoxicity of some peptides at high concentrations [69]. However, distinct composition of mammalian membranes, with preferential localisation of anionic phospholipids into the inner leaflet, offers some protection [69]. These cytotoxic functional properties of HDPs are generally are thought to be associated with their pore-forming activities as multimers in biological membranes leading to self-promoted uptake [111,112] a mechanism that has been further described by the Shia-Matsuzaki-Huang model [113-115]. It is also important to be aware that as HDP hydrophobicity increases, generally there is seen a corresponding increase in antimicrobial activity. However, once hydrophobicity exceeds a certain level, selectivity between prokaryotic and eukaryotic membranes is lost, with a concomitant increase in cytotoxicity [116]. It has been demonstrated in animal models of infection that low dose hCAP/LL-37 therapy gives a significant survival benefit over high dose and control groups. In both studies, high dose hCAP/LL-37 therapy was associated with a higher mortality rate when compared to both low dose and control groups [117,118]. LL-37 has been shown to have non-selective cell toxicity, hemolytic activity and causes DNA fragmentation in cell cultures [119,120]. Many other HDPs were shown to be cytotoxic [120]. These setbacks led to increased efforts to develop novel synthetic HDPs with less cytotoxic effects and improved antimicrobial activity.

The concentrations of HDPs found at many body sites (for example, the mucosa) are inconsistent with a primary role in direct antimicrobial (killing) action. In addition, HDPs show decreased activity at the physiological conditions found in humans due to their sensitivity to both saline solutions and monovalent/divalent ions such as Mg^{2+} or Ca^{2+} in serum [54,121,122]. Hancock *et al.* observed that beta defensin completely loses its activity in concentrations of 100 mM 0.9% NaCl [54]. The weak antimicrobial activity under physiologically relevant conditions has been observed with many HDPs and has led to increasing discussion as to whether the primary function of certain HDPs is to kill bacteria directly [123,124]. Studies have shown that at human physiological conditions HDPs have minimal direct antimicrobial activity but retain their immunomodulatory properties to provide *in vivo* protection [121,122].

6. Are We Entering the Post-Antibiotic Era?

Increased levels of HDPs are often found in inflamed or infected skin areas indicating a role of these peptides in the protection from infection. Several studies have indicated that HDPs have therapeutic potential as topical anti-infectives [73,123-126]. The broad spectrum of antimicrobial activity, the low incidence of bacterial resistance and their function as immunomodulatory agents are attractive features of HDPs for clinical use. Nevertheless, the morbidity of their cytotoxic effects on host cells may limit their use but inform the development of synthetic cationic peptides with diminished cytotoxic profiles and enhanced therapeutic properties, such as the ovispirin (sheep HDP)–derived designer peptide proline-novispirin G10, which provides broad-spectrum antimicrobial activity with low haemolytic and cytotoxic activities [73,125]. Removal of hydrophobic amino acids from the N-terminal end of hCAP18/LL-37 holds promise as a template for the reducing saline sensitivity but maintaining antimicrobial activity [128].

An alternative strategy is gene transfer and the development of target specific HDPs (STAMP = Specific target HDPs); they have already shown new ways to combat infection and inflammation with HDPs *in vitro* and *in vivo* [73,129,130]. Another promising approach is the combined application of two or more HDPs [121] as well as the combination of HDPs with commonly used antibiotics [132] in an effort to prevent the development of antimicrobial resistance and to provide the infected host with optimized antimicrobial therapy [131,132].

The clinical application of HDPs is still in its infancy. However, under the few available clinical studies [133] four peptides demonstrated clinical effectiveness [109]. In 2000 Levin *et al* showed decreased morbidity in children suffering from meningococcal sepsis, who were treated with the designer host defense peptide, rBPI21 (Neuprex), derived from bactericidal/permeability-increasing protein [BPI], a human host defense protein produced by polymorphonuclear leukocytes [134]. Currently, there are ongoing clinical trials investigating therapeutic effects of rBPI21 in burn patients and MSI-78 in diabetic foot ulcers and impetigo.

The bovine indolicin-derived Migenix MX-226 has already proceeded to a phase 3B study for the treatment and prevention of catheter-associated infections [133,109]. Promising candidates for the treatment of acne are the designer peptides XOMA 629 and MBI 594AN with bactericidal effects against *Propionibacterium acnes* [135]. This agent is expected to be available for clinical therapy in the near future.

Table 2. Host defense peptides in commercial development.

Drug	Stage of development	Medical use
BL2060 (a synthetic compound comprising fatty acid and lysine copolymers)	Lead optimization	Anti-infective
CSA-13 (cationic steroid (ceragenin) that mimics host-defense peptides)	Preclinical	Anti-infective
CZEN-002 (synthetic 8-mer derived from -melanocyte-stimulating hormone)	Phase 2b	Vulvovaginal candidiasis
HB-50 (synthetic natural peptide mimetic of cecropin)	Preclinical	Anti-infective
HB-107 (19-amino-acid fragment of cecropin B)	Preclinical	Wound healing
hLF-1-11 (small peptide derived from human lactoferrin)	Phase 2	Allogeneic bone marrow stem cell transplantation-associated infections
IMX942 (5-amino-acid peptide)	Lead optimization	Immunomodulation; treatment of fevers and neutropenia in chemotherapy patients
MSI-78	Phase IIIb	Anti-infective; Wound healing
Omiganan pentahydrochloride/ CP-226/MX-226/CLS001 (12-mer analog of bactolysin)	Phase 3b	Prevention of catheter-related infections; dermatology-related infections
MBI 594AN	Preclinical	Anti-infective
Mersacidin (bacteriocin)	Preclinical	Gram-positive infections
Plectasin (fungal defensin)	Preclinical	Systemic anti-Gram positive, especially pneumococcal and streptococcal infections
PAC113 (based on the active segment of histatin 5 protein found in human saliva)	Investigational New Drug (IND) approval	Oral candidiasis
PTX002 (33-mer peptide) PTX005 (12-mer peptide), PTX006 (N-acylated analog of PTX005) and PTX007 (a nonpeptidic structural analog of PTX005)	Discovery	Broad-spectrum antimicrobial antiendotoxin
Peptidomimetics (derived from the arylamide, calixarene, hydrazide and salicylamide series)	Discovery/preclinical	Anti-infectives; antimicrobial polymers and coating materials
rBPI21	Phase IIIb	Anti-infective; Allogeneic bone marrow stem cell transplantation-associated infections; Prevention of burn infections
XOMA 629	Phase 2a	Anti-infective

7. Conclusions

The emergence of bacterial resistance to antibiotics has led to an increased urgency to explore alternative means of combating pathogenic assault. HDPs are rapidly emerging as attractive candidates for anti-microbial treatment. HDPs have proven to have an important role in host defenses, with many desirable features, and represent an entirely novel class of antimicrobials. The contrasting direct antimicrobial and immunomodulatory activities make these peptides valuable in the design of alternatively directed therapeutic agents and as tools in dissecting the variations in the mechanisms

that underpin these diverse activities. In addition, their small size makes them potentially exciting prototypes for development as novel immunomodulatory drugs, especially since their collective ability to enhance chemokine production, induce chemotaxis, and block endotoxin responses. Future work will involve optimizing these HDPs and better characterizing their immunomodulatory properties to further illuminate their potential as novel therapeutic agents. In addition as HDPs act via a wide acting innate immune system rather than directly on microbe there should not be resistance. There is potential for the clinical success of antimicrobial HDPs. And although they do not solve all of the issues created by the problem of antibiotic resistance, they do offer a prospectively exciting new tool in the clinician's armamentarium.

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