Airway epithelial innate host defence in chronic obstructive pulmonary disease

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CHAPTER 10

Antimicrobial peptides and innate lung defenses: role in infectious and non-infectious lung diseases and therapeutic applications.

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ABSTRACT

Respiratory infections are a major clinical problem, and treatment is increasingly complicated by the emergence of microbial antibiotic resistance. Development of new antibiotics is notoriously costly and slow, and therefore alternative strategies are needed. Antimicrobial peptides, central effector molecules of the immune system, are being considered as an alternative to conventional antibiotics. These peptides display a range of activities, including not only direct antimicrobial activity but also immunomodulation and wound repair. In the lung, especially airway epithelial cells and neutrophils contribute to their synthesis. The relevance of antimicrobial peptides for host defense against infection has been demonstrated in animal models, and is also supported by observations in patient studies, showing altered expression and/or unfavorable circumstances for their action in a variety of lung diseases. Importantly, antimicrobial peptides are active against micro-organisms that are resistant against conventional antibiotics, including multidrug resistant bacteria. Several strategies have been proposed to use these peptides in the treatment of infections, including direct administration of antimicrobial peptides, enhancement of their local production and creation of more favorable circumstances for their action. In this review, recent developments in antimicrobial peptides research in the lung and clinical applications for novel therapies of lung diseases are discussed.

INTRODUCTION

Respiratory infections are a major clinical problem. According to the World Health Organization (WHO), lower respiratory infection was the fourth leading cause of death worldwide in 2012 (1). In addition to pneumonia and bronchitis, lower respiratory tract infections also markedly contribute to chronic inflammatory lung disorders such as cystic fibrosis (CF), chronic obstructive pulmonary disease (COPD) and asthma. Although antibiotic treatment is considered as the most effective medical intervention currently available, there is a growing concern regarding the increase of microbial antibiotic resistance. Antibiotic resistance is associated with marked morbidity and mortality, and also poses an increasing economic burden. Therefore, it is now considered as a top public health threat for which novel approaches are needed. In addition to stimulating the prudent use of antibiotics, there is an urgent need for new antibiotics. However, it has proven increasingly difficult and extremely costly to develop novel antibiotics resulting in a limited pipeline for new antibiotics. Therefore, many pharmaceutical companies have put a hold on their antibiotic research activities.

Several alternatives for antibiotics have been proposed, and in a back-to-nature approach new strategies are being developed, including the use of bacteriophages, probiotics and antimicrobial peptides. Antimicrobial peptides (AMPs) are effector molecules of the immune system, and research has demonstrated that these peptides may serve as potential alternatives for conventional antibiotics. AMPs display broad-spectrum antimicrobial activity against bacteria, fungi and viruses, including multidrug resistant (MDR) micro-organisms and those present in biofilms. Production of AMPs by e.g. airway epithelial cells and neutrophils is one of the varieties of mechanisms used by the lung to deal with the continuous largescale exposure to numerous inhaled pathogens. In this review we will discuss the natural role of AMPs in lung host defense and their therapeutic potential in the treatment of lung diseases.

ANTIMICROBIAL PEPTIDES AND THEIR ROLE IN HOST DEFENSE AGAINST RESPIRATORY INFECTIONS AND IN CHRONIC LUNG INFLAMMATION

Originally discovered for their (direct) antimicrobial actions, AMPs are nowadays increasingly recognized for their miscellaneous qualities ranging from antimicrobial to anti-biofilm, anticancer and immunomodulation. AMPs are part of the evolutionary conserved innate immune system, and are abundantly produced in the lung and other mucosal tissues, where they act as the first line of defense against infections (2) . Over 2000 naturally occurring AMPs have been identified so far (http://aps.unmc.edu/AP/main.php), and also humans express various AMPs. These AMPs have overlapping actions, but often are active against different pathogens, at different locations in the body with different mechanisms of action. The main AMPs that are detected in lung tissues and secretions, are neutrophil α-defensins/human neutrophil peptides (HNPs), human β-defensins (hBDs) and the cathelicidin hCAP18/LL-37 (3, 4). In the lung, not only the smaller AMPs but also other larger antimicrobial proteins are produced, including lysozyme, lactoferrin, secretory leukocyte proteinase inhibitor (SLPI), elafin and RNase 7 (5) . Several cell types contribute to production of AMPs in the lung. Airway epithelial cells represent a major source of these peptides in the lung, producing e.g. hBD-1, hBD-2, hBD-3 and LL-37. These AMPs are expressed either constitutively or are

induced by microbial exposure, injury, cytokines, growth factors produced during wound repair, or (micro)nutrients such as vitamin D 4 . Also, myeloid cells such as neutrophils and macrophages, contribute to the presence of AMPs in the lungs. In particular, neutrophils that are attracted during inflammation are the primary source of HNPs and LL-37. These AMPs act intracellular within the phagolysosome to kill ingested micro-organisms, or are secreted or released in complex with DNA in structures known as neutrophil extracellular traps (NETs) (6). Recent studies highlight that AMPs such as LL-37 may also stimulate formation of NETs and stabilize these by providing protection against nuclease-mediated degradation, and thus contribute to this recently identified mechanism of host defense (6, 7).

The presence of these AMPs contributes to innate lung defense by displaying a variety of mechanisms of action, including direct antibacterial and antifungal activity mediated by lysis of micro-organisms through formation of transmembrane pores or by impairing bacterial viability by affecting processes such as cell wall biosynthesis (8). In addition, AMPs display antiviral activity against a range of viruses (9). Besides their direct antimicrobial activity, AMPs have an active role in shaping the immune response in the lung, displaying pro- and anti-inflammatory properties, chemotactic activity, inducing expression of cytokines and chemokines and modulating dendritic cell maturation (10). Moreover, they can promote wound healing by enhancing airway epithelial cell repair and promoting angiogenesis (11, 12). The detailed contribution of AMPs to innate defenses against infections has been clearly demonstrated in a variety of animal model studies. In the fruit fly Drosophila melanogaster, which only has an innate immune system, abrogation of microbial induced expression of AMPs resulted in enhanced infections and reduced host survival (13). In line with this, aerosol treatment with a bacterial lysate or a combination of microbial ligands, provides protection against a broad-spectrum of respiratory pathogens in a murine model (14). This protection was not dependent on immune cells, but mediated by lung epithelial cells through induced expression of AMPs. An example of the importance of microbial induced expression of AMPs in host defense is observed in patients with Crohn's disease that have a polymorphism in the gene encoding the cytosolic microbial pattern recognition receptor nucleotide-binding oligomerization domain-containing protein 2 (15). This polymorphism impairs intestinal expression of AMPs, thereby contributing to enhanced infections and chronic inflammation.

Whereas these studies highlight the relevance of AMPs for host defense in general, a variety of studies also provide direct evidence for a protective role of AMPs in lung infections. This is well-illustrated by research on the cathelicidin peptide LL-37 and the cathelicidin mouse orthologue (cathelicidin-related antimicrobial peptide) CRAMP. Transgenic overexpression of LL-37 in mice provided protection against Pseudomonas aeruginosa infection (16). Conversely, CRAMP deficient mice were more sensitive to Gram-negative bacterial pneumonia (17). Similar in vivo studies also support a role for human LL-37 and murine CRAMP in host defense against influenza A respiratory viral infections (18). Moreover, in individuals with vitamin D deficiency, reduced expression of LL-37 expression may increase susceptibility to tuberculosis infections (19). Similar model studies have highlighted the role of e.g. β-defensins in respiratory infections (reviewed in (20)).

In addition to a role in respiratory infections, aberrant expression and activity of AMPs is also associated with non-infectious lung diseases (Table 1). Airway epithelial expression of hBD-

2 is reduced in smokers, patients with COPD, and CF lung disease, which could be a reason for increased microbial colonization and infections in these diseases (21-23). Also allergic airways inflammation has been shown to reduce AMP expression (24). Moreover, reduced pH of the airway surface liquid as observed in infants with CF, may impair the activity of AMPs, as demonstrated in a porcine CF model and in vitro (25, 26). Importantly, not only the pH of the local environment but also other local conditions may impair the antimicrobial activity of AMPs, including AMP degradation by microbial and host proteases (27, 28), and inhibition of AMP activity by salt, microbial polysaccharides, F-actin and DNA from dying cells, and mucus (29).

Table 1. AMPs and their relevance for lung diseases

AMP	**Localization (main cell types)**	**Changed expression in lung disease**
Neutrophil α-defensins (Human neutrophil peptides [HNP]1-4)	Neutrophils	• Asthma: increased systemic expression of HNP-1 in especially neutrophilic asthma (58) • Asthma: increased BAL HNP1-3 levels in RV infection (34) • Bronchiolitis obliterans syndrome: increased HNP1-3 in BAL(33) • COPD: HNP-1 and HNP-2 increased in BALF(31); sputum HNP1-3 associated with COPD severity (30) • Cystic fibrosis: high levels of HNP1-3 in sputum (59)
β-defensins	Mainly airway epithelial cells	• Acute pneumonia: smoking associated with reduced hBD-2 in pharyngeal washings (22) • COPD: Increased hBD-1 expression in central airways in COPD (60) • COPD: hBD-2 expression is decreased in central airways (21) and sputum and BAL(61), and increased in peripheral lung tissue(62) • Cystic fibrosis: decreased hBD-2 levels correlate with disease severity (23) • Diffuse panbronchiolitis: increased hBD-2 in plasma and hBD-1 and hBD-2 in BAL(63)
hCAP18/LL-37	Neutrophils and airway epithelial cells	• Bronchiolitis obliterans syndrome: increased levels in BAL(33) • COPD: increased expression in small airways in smokers and especially smokers with COPD(64) • Cystic fibrosis: increased levels in BAL correlate with disease severity(23) • Lung cancer: increased expression(65)

In contrast to the impaired expression and activity of airway epithelial AMPs in COPD and CF, increased neutrophilic inflammation in these diseases is associated with increased levels of neutrophil-derived HNPs and LL-37 (23, 30, 31). Moreover, exacerbations in COPD caused by bacterial infections, or induced experimentally upon rhinovirus infection, are associated with further increased levels of neutrophil-derived AMPs in the lung (28, 32). Similar to

COPD and CF, increased neutrophilic inflammation correlated with enhanced levels of neutrophil-derived AMPs in neutrophilic asthma, bronchiolitis obliterans syndrome, and interstitial lung disease (33-35). In these diseases, especially neutrophil-derived AMPs might contribute to lung injury and inflammation through their pro-inflammatory activity and cytotoxic properties at high concentrations. These studies illustrate that both insufficient as well as excessive levels of AMPs may contribute to lung disease development and progression. Low levels of AMPs or impairment of their activity may increase susceptibility to infection, alter the microbiome and impair wound repair, inflammation and immunity. In contrast, highly increased levels of AMPs may contribute to inflammation, uncontrolled immune cell recruitment, tissue injury and an altered microbiome. This illustrates that homeostasis of AMPs in the lung is important and that lack or excess of AMPs both have detrimental effects. Therefore, monitoring levels of AMPs in respiratory secretions or tissue in inflammatory lung diseases may provide important information on disease pathogenesis. Furthermore, when designing clinical trials using AMPs, these consequences of inappropriate levels of AMPs need to be taken into consideration.

AMPS AS CANDIDATES FOR DRUG DEVELOPMENT

Use of AMPs as therapeutics seems a valid option based on their multiplicity of actions against multidrug resistant (MDR) pathogens as discussed above. Furthermore, their antibiofilm qualities are extremely relevant in a hospital setting where biofilms (including those with MDR micro-organisms) on both biotic surfaces (such as the airways) as well as on implants or mechanical ventilators are notoriously therapy resistant (36). However, because of the variety of antimicrobial and other activities, the therapeutic window of these peptides is probably different for each peptide and each disease. As discussed in the previous section and illustrated in Figure 1, a relative deficiency as well as excessive AMP expression and/or activity may be detrimental. Several factors need to be taken into account that could affect the therapeutic success of AMP-based therapeutic strategies: 1) type of AMP used, 2) route of administration and 3) local environment. However, a threat to this use is the potential development of pathogenic resistance against these peptides (37). Especially the development of resistance against endogenous peptides would be detrimental for the host defense against infection. However, as AMPs are ancient molecules that have diverse actions, complete resistance as detected in modern antibiotics is not expected to occur (38). Another potential risk could be interference of externally administered AMPs with microbiota. This is important since the composition of the microbiome is highly relevant to the development and progression of chronic lung disease (39). However, especially since the microbiome also needs to be protected against unwanted actions of endogenous AMPs, it may not be surprising that the healthy microbiome appears to be relatively protected against AMP-mediated killing (40).

Proof-of-principal for therapeutic efficacy of endogenous AMPs in lungs has been delivered by over-expressing LL-37 in the lungs of mice or by direct administration of this peptide to the lungs. This resulted in reduced bacterial load and enhanced survival of the infected mice (16, 41). AMPs were also active against P. aeruginosa in a rat model of CF where both reduced pathogenic load and anti-inflammatory activity was observed by these peptides (42). Alternatively, one could rebalance endogenous production of AMPs, for instance when

expression of these are compromised during disease. Several inducers of endogenous AMP expression have been discovered over the last 10 years, of which sodium- and phenylbutyrate have shown promising activities in clinical trials (43, 44). The success of vitamin D treatment, another inducer of AMP expression, may be most pronounced in those patients with severe vitamin D deficiency (45, 46) .

Despite the array of naturally occurring AMPs, little progress has so far been made with therapeutic development of these endogenous peptides. This may in part be explained by AMPs not displaying optimal activity in an inflamed and infected lung. Especially in CF, the loss of function of the cystic fibrosis transmembrane conductance regulator (CFTR) has multiple consequences that may affect endogenous AMP activity, including direct effects on the pH in the airway surface liquid as mentioned before and indirect effects caused by inflammation, cell death and mucus hypersecretion. Therefore, the discovery of small molecules that restore CFTR function may have important implications for local AMP activity in the lungs of CF patients treated with such compounds. Furthermore, in the lung a rather different environment exists compared to for example skin, gut or tissue. So peptides possibly have to be designed for the specific microenvironment they have to be functional in to withstand protease activity or for example unfavorable pH. Furthermore, the local environment can also alter AMP activity (47), which is another feature that needs to be taken into consideration as AMPs might work in one organ and not in another.

The limited success of AMP-based therapy so far may also be explained by the fact that these AMPs have several disadvantages for therapeutic use in addition to the loss of local activity, such as a short half-life and toxicity. An alternative and perhaps also complementary strategy is the development of improved substitutes for AMPs. Innate defense regulators (IDRs) are a group of synthetic peptides inspired by various naturally occurring peptides with the aim to enhance their immunomodulatory activity for therapeutic use (48). So far, IDR peptides were shown to reduce pathogen load and inflammation in mice infected with (MDR) M. tuberculosis (49) and were successful as a vaccine strategy in mice and cotton rats against RSV (50). Furthermore, research also focuses now on alternative compounds based on AMPs (e.g. peptidomimetics) for exogenous administration. Peptidomimetics are compounds that have favorable qualities compared to AMPs, though at lower production cost and without protease sensitivity (51). Both in vitro and in mice, AMPs mimetics have been shown to have favourable effects against for example oral candidiasis (51, 52) .

In addition to the component itself, the route of administration needs to be wisely chosen also taking into consideration which activity of the AMP is targeted. When aiming for direct antimicrobial activity, most likely inhaled administration is preferred with these peptides over systemic administration. The immunomodulatory effects of AMPs seem more robust and could probably also contribute to their activity upon systemic administration, if toxicity allows this type of delivery. Administration can be further enhanced for example by combining AMPs with exogenous surfactant, which was demonstrated to be a promising possibility for improved delivery of AMPs to the lung (53). Alternatively, also for the inducers of endogenous AMPs, their effectiveness can be improved by enhancing their delivery (54). Lastly, combining AMPs with antibiotics shows promising results in the fight against (MDR) microorganisms as synergy is observed in their antimicrobial actions (55, 56). This synergy

may in part be explained by the ability of AMPs to increase bacterial membrane permeability to antibiotics, as recently demonstrated by the ability of LL-37 to potentiate the penetration of azithromycin into MDR bacteria (57) .

Altogether, successful management of an AMP(-based) therapy seem challenging due to the high variety of factors that need to be taken into consideration. However research shows innovative solutions to optimally target AMPs for therapeutic success. Whereas in the past, several clinical trials have evaluated the effect of AMP administration for the treatment of respiratory diseases, the current focus in ongoing clinical trials is on treatments, which induce the endogenous expression of AMPs (www.clinicaltrials.gov).The encouraging trend observed in e.g. studies with vitamin D and phenylbutyrate will hopefully show promising results further stimulating research into this direction.

CONCLUSION

Research on AMPs has flourished in the past decades, but so far has not resulted in major breakthroughs in the treatment of respiratory infections. This is partly explained by the low cost and effectiveness of conventional antibiotics for the majority of patients. As a result, new antibiotic strategies are usually reserved for patients with complicated infections, and as a result the potential market is still limited. In this review we have highlighted why AMPs may be interesting candidates for novel antibiotic strategies, and shown that the multitude of activities displayed by AMPs may both be an advantage of their use, but also poses specific challenges. There are three promising strategies for their use: direct application of AMPs or AMP-inspired compounds, enhancement of local production and improving local conditions for AMPs actions (Figure 1). In addition, further research on the mechanisms underlying

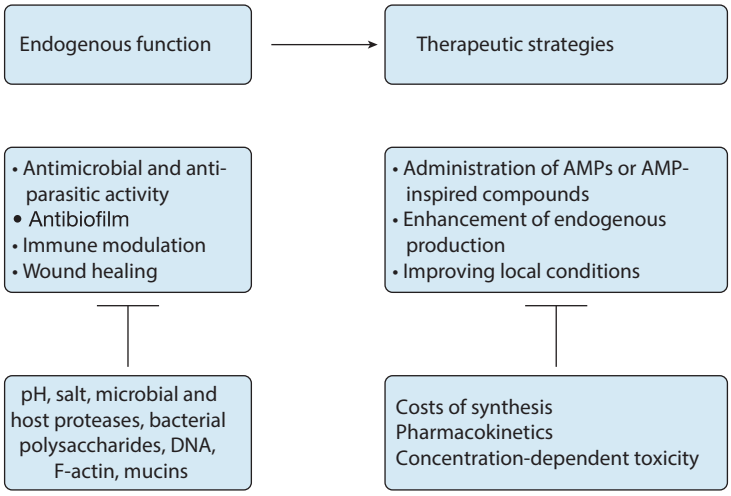

Figure 1. Endogenous functions and possible therapeutic strategies for using AMPs in the treatment of infectious or inflammatory lung diseases. Although AMPs offer an attractive alternative to conventional antibiotics, various factors that may limit their use need to be considered. AMPs = antimicrobial peptides.

deficient AMP expression and/or activity in chronic lung diseases is needed, and may also lead the way to the discovery of novel treatments.

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