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Review

Therapeutic Role of Antimicrobial Peptides in Diabetes Mellitus

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Abstract: Antimicrobial peptides (AMPs) have recently become widely publicized because they have the potential to function in alternative therapies as "natural" antibiotics, with their main advantage being a broad spectrum of activity. The potential for antimicrobial peptides to treat diabetes mellitus (DM) has been reported. In diabetes mellitus type I (T1D), cathelicidin-related antimicrobial peptide (CRAMP), cathelicidin antimicrobial peptide (CAMP) and mouse-β- defensin 14 (mBD14) are positively affected. Decreased levels of LL-37 and human neutrophil peptide 1-3 (HNP1-3) have been reported in diabetes mellitus type II (T2D) relative to healthy patients. Moreover, AMPs from amphibians and social wasps have antidiabetic effects. In infections occurring in patients with tuberculosis-diabetes or diabetic foot, granulysin, HNP1, HNP2, HNP3, human beta-defensin 2 (HBD2), and cathelicidins are responsible for pathogen clearance. An interesting alternative is also the use of modified M13 bacteriophages containing encapsulated AMPs genes or phagemids.

Keywords: antimicrobial peptides; diabetes mellitus; cathelicidins; diabetic foot; tuberculosis

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1. Introduction

Antimicrobial peptides (AMPs) are small peptides with a broad spectrum of activity. They are known for their antimicrobial and immunomodulate properties against Gram-positive and Gram-negative bacteria, viruses and fungi. AMPs are divided into classes based on classification criteria such as net charge, secondary structure and solubility [1]. There are also other classifications, including aminoacids sequences, their structure and other characteristics [2]. Cationic, alpha-helical AMPs are the most widely distributed AMPs in the environment in the environment. They are capable of disrupting cell-membrane integration, which leads to osmotic shock and death. AMP are not prone to developing resistance and are responsible for the innate immune response, creating a rapid first line of defense against infection [3].

Diabetes mellitus (DM) is a heterogeneous metabolic disease, classified as a civilization disease. DM is a heterogeneous metabolic disorder characterized by the presence of hyperglycemia due to impairment of insulin secretion, defective insulin action or both. The chronic hyperglycemia of DM is associated with relatively specific long-term microvascular complications affecting the eyes, kidneys, and nerves and an increased risk for cardiovascular disease (CVD) [4]. There are two types of DM–type I (T1D) and type II (T2D) [4].

During DM, diabetic foot syndrome can develop, as a result of long-term hyperglycemia, as hyperglycemia leads to damage to the blood vessels and results in their reduced elasticity, overgrowth and increased atherosclerosis. Tissue ischemia leads to disorders, thrombus formation, tissue hypoxia and infection with common bacteria such as *Staphylococcus aureus* and *Enterococcus faecalis* [5].

Tuberculosis (TB) is caused by the bacteria–*Mycobacterium tuberculosis* (MTB), and 25% of the diabetic population is infected [6]. Mostly it attacks the lungs; other organs are usually targeted by zoonotic bacteria–for example, *Mycobacterium bovis*. Patients infected with MTB can develop active disease, resistance to disease, clearance of infection or latent infection without syndromes [6]. Patients with TB and DM co-morbidity often develop cavitary lung lesions and have four-fold chances of relapse, which is associated with increased mortality [7]. Increased multi-drug and extensive drug resistance has been observed in both DM and non-DM TB patients. This means that new forms of treatment are needed [8]. In this work, we investigated the possibilities of using AMPs in people with DM and coexisting diseases. We consulted PubMed and Google Scholar using the core search words 'AMP', 'AMP diabetes', 'diabetic foot infection', 'AMP characteristic', 'AMP wound healing', 'AMP tuberculosis', 'tuberculosis diabetes'.

2. AMPs Characteristics

AMPs were discovered in 1939 by Dubos [9], who isolated an antimicrobial compound from a soil strain of *Bacillus* that protected mice from pneumococcal infection. A year later, it was named gramicidin [10]. The first AMP of animal origin is defensin. In 1956, it was isolated from rabbit leukocytes [11]. In the following years, bombinin was also discovered in the epithelium [12], lactoferrin in cow's milk [13], and AMP in the lysosomes of human leukocytes [14]. To date, 5000 AMPs have been discovered and synthesized. They play an important role in fighting infection before any symptoms appear, so naturally, in animals, AMPs are found in tissues and organs that are most vulnerable to infection [15].

AMPs are produced by various cell types, including gastrointestinal and genitourinary epithelial cells [16], phagocytes [17], lymphocytes [18] or hemocytes [19]. They can be produced continuously or induced by infectious or inflammatory stimuli such as pro-inflammatory cytokines, bacteria, or bacterial particles (e.g., lipopolysaccharides) [20]. Some AMPs, such as 18-kDa cationic antimicrobial protein (CAP18), 35-kDa cationic antimicrobial protein (CAP35), and a lactoferrin derivative, may contribute to reducing the inflammatory response by inhibiting lipopolysaccharide (LPS)-induced cytokine release [21–23].

In mammals, we can observe two classes of AMPs: cathelicidins and defensins [24]. Cathelicidins have different peptide lengths, protein structures, and a highly conserved cathelicidin domain [25]. The only cathelicidin found in humans is LL-37. It has been shown to neutralize LPS, inhibit macrophage pyroptosis, enhance the release of neutrophil extracellular traps (NETs), and stimulate neutrophils to release antimicrobial microvesicles (ectosomes) [26]. Another class of AMPs found in humans, the defensins, are divided into two subgroups: α-defensins and β-defensins [27,28]. Vertebrate defensins are synthesized as prepeptides that require proteolytic processing to their active peptide forms. Although the bactericidal activity of defensins is low, high concentrations of α-defensins in phagocytic cell granules and intestinal crypts are sufficient to combat pathogens [20].

Most antimicrobial polypeptides can be divided into five subgroups based on their amino acid sequences: (I) α-helix, (II) β-sheet, (III) anionic, (IV) extended cationic and (V) the last subgroup is the fragments from antimicrobial proteins [24]. The cationic α-helical peptides are the best known and most common AMPs. The distance between two adjacent amino acids in this group is about 0.15 nm [29]. α-helices are amphiphilic peptide molecules; that is, they contain hydrophobic and hydrophilic halves [30]. This subgroup includes small peptides less than 40 amino acids in length with a net charge of +2 to +9. They predominantly have an amidated C-terminus [31]. The best-studied representatives of this group are protegrin, magainin, cyclic indolicin and coiled indolicin [31]. The second most common structure is β-sheet, which consist of at least two β-strands linked by disulfide bonds [32]. They contain two to eight cysteine residues. β-sheet AMPs are composed mainly of defensins [33]. The third subgroup is anionic AMPs with a net charge of -1 to -8. They contain from 5 to 70 amino acid residues [34]. This group includes mainly peptide fragments after proteolysis and less numerous small molecules encoded by

genes. They interact with microorganisms by forming salt bridges using metal ions and negatively charged components of the microbial cell membrane [35]. The fourth subgroup, extended cationic AMPs, are linear without cysteine residues and contain specific amino acids (arginine, proline, tryptophan, glycine, histidine) [36]. Their structures are stabilized only by hydrogen bonds and van der Waals force of interaction with membrane lipids [24]. The last subgroup are fragments of some naturally occurring antimicrobial proteins that have bactericidal activity. A representative of this group is the helix-loop-helix (HLH) peptide found in human lysozyme, which has bactericidal activity against Gram-positive and Gram-negative bacteria and the fungus *Candida albicans* [24].

A key attribute of AMPs is that their target is directed at the LPS layer of the microbial cell membrane. Eukaryotic cells, due to their high cholesterol and low anionic charge, are off target for most AMPs [20]. Due to their positive charge, AMPs react with negatively charged cell membranes through electrostatic interactions [30]. Another feature of AMPs is their ability to kill within seconds of initial contact with the cell membrane [37].

2.1. AMPs Modifications

Some AMPs must be post-translationally modified to perform their functions or to improve their stability, activity and desirability. The variety of structural scaffolds of physical AMPs may enable them to recognize different cellular targets such as cell walls, membrane proteins and nucleic acids [38]. AMP activity is affected by many factors such as peptide length, net charge, hydrophobicity, and secondary structure [24].

2.1.1. Modification by Covalent Bonds

Disulfide bonds are an important component of many different AMPs. It has been shown that covalent modifications can significantly affect the structure and function of AMPs [38]. For example, adding a disulfide bond to CP-11 does not affect antimicrobial activity, but increases the stability of the structures [39]. In contrast, removing the disulfide bond in protegrin results in a lack of activity against HSV [40].

2.1.2. Modification by Changing the Amino Acid Content

Amino acid modification is the best-studied method for altering AMP function. This method is based on the physiological characteristics of specific amino acids that affect the spectrum of peptide action. The ability of AMPs to penetrate microbial cell membranes is affected by proline. For example, increasing the proline content of CP26 decreases this ability against *Escherichia coli* [41]. In contrast, amino acids such as asparagine and glutamine affect the cytotoxic properties of AMP. Their removal from LL37 and the addition of arginine results in reduced cytotoxic effects on eukaryotic cells [42].

2.1.3. Modification by Amidation

Modification by amidation involves the addition of chemical amide groups at the end of peptides. For example, the amidation of PMAP-23 changes its orientation inside the cell membrane to perpendicular, which results in faster interaction with the cell membrane of Gram-positive bacteria and deeper insertion into the inner membrane [43]. This effect is not universal because not all amidated peptides show increased activity after amidation. For example, replacing the amide group at the C-terminal with a free acid may contribute to greater stability of the derivative, as in the case of Api88 [44].

2.1.4. Modification by Combining Two AMPs

It has been shown that modification by the combination of two AMPs can cause increased antimicrobial activity. For example, the interaction between polymyxin B (PMB) and gramicidin S (GS) resulted in more effective activity against biofilms of *Pseudomonas aeruginosa* strain PAO1. It is supposed that PMB reacts with LPS of Gram-negative bacteria and facilitates the translocation of GS across the bacterial cell membrane. This mechanism results in increased bactericidal activity against biofilm-forming cells [45]. However, there

is no information available on the effect of combining two AMPs on toxicity to the host. Therefore, synthesizing AMPs from key residues derived from two to three peptides with different mechanisms of action seems to be a promising prospect. For example, the synthesized H4 peptide formed from the combination of two single α-helical fragments, BMAP-27 and OP-145, showed a broad spectrum of action and low toxicity [46]. Another synthesized peptide being a hybrid of cecropin-A, melittin and LL-37, exhibited increased antibacterial activity against Gram-positive and Gram-negative bacteria and decreased hemolytic activity [47].

3. Role of AMPs in T1D

T1D is a chronic, autoimmune disease caused by the damage of insulin-producing β- cells in pancreatic islets [48–50]. The lack of properly functioning β-cells leads to a decrease in the amount of produced insulin, which disrupts the proper functioning of the gastrointestinal tract. Gastrointestinal complications of T1D include gastroparesis, intestinal enteropathy, and nonalcoholic steatohepatitis. They are most commonly caused by abnormal gastrointestinal motility, which is a consequence of diabetic autonomic neuropathy involving the gastrointestinal tract [51]. Treatment of patients with T1D includes lifelong exogenous insulin doses [49]. In T1D, β-cells are destroyed when T cells recognize autoantigens such as proinsulin, islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP), glutamic acid decarboxylase (GAD) and islet cell antigen 69 (ICA69) [50]. However, β-cells located in the pancreas have more than one function. In addition to producing insulin, they are also responsible for the production of cathelicidin-related antimicrobial peptide (CRAMP) [48,49,52]. This implies that patients who are suffering from T1D also have reduced amounts of this AMP compared with healthy people [49,52]. A 2015 study found that the production of CRAMP in non-obese diabetic (NOD) mice is defective and administration of CRAMP to pre-diabetic rodents induces the regulation of immune cells in diabetic islets, thereby reducing the incidence of autoimmune diabetes. In addition, it was established that the production of CRAMP is regulated by short-chain fatty acids derived from gut microbiota [52].

Cathelicidin antimicrobial peptide (CAMP), the human CRAMP homolog, also plays an important role in susceptibility to T1D. CAMP is expressed in B cells, where it stimulates several islet pathways such as glucose sensing and hormone secretion. A 2015 study on diabetes-prone BBdp rats found that CAMP has a positive effect on the functioning and regeneration of pancreatic islets. Additionally, it has been shown that CAMP can act as both an autocrine and a paracrine factor stimulating the secretion of both insulin and glucagon and thus can act as a regulator of intra-island communication [53].

Another AMP that can affect T1D is mBD14 is mouse-β- defensin 14 (mBD14), an AMP whose expression is induced by innate lymphatic cells in the pancreas. Mouse-β-defensin 14 stimulates Toll-like receptor 2, interleukin-4 secreting β cells, that induce regulatory macrophages, which induce protective regulatory T cells. According to a 2018 study conducted on mice suffering from NOD, it was found that treatment with mBD14 prevents autoimmune diabetes [49,54].

4. Role of AMPs in T2D

T2D is a metabolic disease characterized by elevated blood glucose levels. It is accompanied by phenomena such as insulin resistance, β-cell dysfunction, and increased glucose secretion by the liver. One treatment for T2D is pharmacological manipulation of the AMP-activated protein kinase (AMPK) activation pathway, which results in increased cellular sensitivity to insulin [55]. This information suggests that AMPs may be involved significantly in both the development and treatment of the disease. A study by Zainab and co-workers [56] showed that patients with T2D had significantly lower levels of LL-37 and HNP 1-3 (neutrophil antimicrobial peptides) compared to patients without T2D. Although there are few studies on the role of AMPs in the development of T2D, the prospects for using these peptides to treat the disease look promising. An informatics review and analysis

by Soltaninejad et al. [57] identified 45 AMPs isolated from amphibians and two from social wasp *Agelaia pallipes* that have antidiabetic activity. However, to date, there are only a few studies in animal models that have looked at the antidiabetic effect of these peptides [58,59]. Nevertheless, these studies showed that the tested peptides exhibited antidiabetic effects at low concentrations and were not toxic to cells. This direction of research gives high hopes for finding effective complementary drugs for T2D. However, further work should focus not only on the antidiabetic effect itself but also on increasing AMPs activity which was low during in vivo studies [57].

5. Role of AMPs in Diabetic Foot Infection (DFI)

As the incidence of DM increases, the risk of developing chronic complications of this disease increases. One of the serious consequences of persistent hyperglycemia is the development of diabetic neuropathy. The risk of developing peripheral neuropathy increases by 10–15% for every 1% increase in glycosylated hemoglobin. Typical symptoms of worsening diabetic neuropathy in the feet are burning, pain and an abnormal feeling of cold and heat [60]. As a result of the damage to the sensory nerve fibers, the perception of pain disappears, so minor and larger cuts do not cause any discomfort and may go unnoticed by the patient for a long time. Injuries occur more easily because motor neuropathy leads to a change in the shape of the foot, causing patients' shoes to no longer fit. Patients often do not notice this because they do not feel the discomfort associated with wearing poorly fitted shoes [61]. In a study by Kumoniewski et al. [5], 66% of patients admitted to the hospital with severe purulent necrotic lesions reported that they initially had minimal epidermal abrasion. These were mainly around the metatarsophalangeal joint of the toe and were caused by cuts, stepping on sharp objects or from cutting a nail too deeply. A delay in admitting to hospitals increases the advancement of necrotic and purulent lesions. The accompanying neuropathy, atherosclerosis of the arteries of the lower extremities, arterial hypertension, hypercholesterolemia and increased sensitivity of the foot skin to damage may lead to the development of diabetic foot syndrome (DFS) [60].

DFS includes ulceration (damage to the skin and/or tissue) below the ankle and infections in the soft tissue or bone. The rupture of the skin allows for the colonization of the subcutaneous tissue by microorganisms, which in many cases results in infection and consequently antibacterial treatment and even surgical treatment [62]. Ischemia caused by atherosclerotic lesions in the arteries of the lower extremities is the primary factor that increases the risk of DFS with concomitant neuropathy. As a result of chronic limb ischemia, wounds take longer to heal, and drugs do not reach infected areas, so antibiotic therapy is ineffective. Due to the symptoms of ischemia, DFS is categorized into neuropathic, ischemic and neuropathic-ischemic forms. The reduction in immunity or immune disorders in patients is also mentioned as a factor of DFS. In ulcers, gangrene and necrosis may occur as a result of the rapidly progressing infection. Commonly used antibiotics penetrate very poorly into ischemic areas, and the use of external antibiotics may only contribute to the selection of resistant bacterial strains [60].

The most common (52.5% of respondents) causes of DFS are infections. Multiple infections significantly complicate the treatment and increase its costs. The most commonly grown pathogen from purulent necrotic lesions in patients with DFS is *Staphylococcus aureus*, followed by *Enterococcus faecalis* [5].

Infection of previously untreated DFS is caused by Gram-positive bacteria, while chronic and severe infections are caused by a mixture of aerobic and anaerobic Gram-negative bacteria, including *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus* sp. Wounds involving deep tissue or ischemic necrosis are attacked by obligate anaerobes. *Pseudomonas* sp. infections are common in wounds soaked in wet dressings and are often found in warmer regions [63]. One of the necessary stages of treatment is, therefore, antibiotic therapy. Initially, it is empirical antibiotic therapy, and after obtaining a microbiological test, targeted antibiotic therapy [5]. Occasionally, as in the case of other chronic infections, a bacterial biofilm may form, which has the ability to survive in unfavorable conditions for

a long time. It is estimated that biofilm is up to 1000 times more resistant to conventional antibiotics [64].

Due to the development of antibiotic resistance, which delays treatment, makes it less effective and endangers the human population, alternatives to antibiotic therapy are being sought. A promising alternative for infected wounds is the use of AMPs, which are active against a wide range of Gram-positive and Gram-negative bacteria. Additionally, some AMPs exhibit immunomodulatory and angiogenic properties, neutralize bacterial toxins, stimulate cell proliferation and migration, inhibit pro-inflammatory reactions and biofilm formation processes, as well as angiogenic properties and accelerate wound healing [65,66]. The action and origin of selected AMPs are presented in Table 1.

Table 1. Selected AMPs and their effects [65–74].

AMP	Origin	Natural Species and the Species on Which the Modified and the Synthetic Peptides Were Based	Activity and/or Effect
α-defensin HD5	Natural	Human: *Homo sapiens*	Eradication of disease caused by infection with *Salmonella typhimurium* and *Staphylococcus aureus.*
α-defensin HNP-2	Natural	Human: *Homo sapiens*	The highest bactericidal activity against *Staphylococcus aureus.*
α-defensin HNP-4	Natural	Human: *Homo sapiens*	The strongest activity against *Escherichia coli* and *Enterobacter aerogenes.*
β-defensin DEFB118	Natural	Human: *Homo sapiens*	Destroys *Escherichia coli* within 15 min.
β-defensin TAP	Natural	Animal: *Mammalia*	Activity against *Echerichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus.*
θ-defensin RTD-1	Natural	Animal: *Macaca*	Activity against *Escherichia coli.*
CW49	Natural	Animal: *Odorrana grahami*	Promote angiogenesis while preventing excessive anti-inflammatory response (tested in diabetic foot ulcer).
Tachyplesin	Natural	Animal: *Limulidae*	Broad-spectrum activities against both gram-positive and gram-negative bacteria; strong biocidal activity against resistant strains of bacteria (*Escherichia coli*, *Staphylococcus aureus*).
Cathelicidin-AM	Natural	Animal: *Ailuropoda melanoleuca*	Activity against *Staphylococcus aureus.*
Esculentin-1a(1-21)	Natural	Animal: *Rana esculenta*	Wound-healing promoter, especially against chronic, often *Pseudomonas*-infected skin ulcers.
Temporins A and B	Natural	Animal: *Rana temporaria*	Promote in vitro wound-healing in a monolayer of immortalized human keratinocytes (HaCaT cells); both temporins can reduce the number of *Staphylococcus aureus* bacteria inside HaCaT keratinocytes.
Saha-CATH5	Natural	Animal: *Sarcophilus harrisii*	Activity against many species of gram-positive and gram-negative bacteria, especially vancomycin-resistant (VRE) and methicillin-resistant *Staphylococcus aureus* (MRSA).
MsDef1 and MtDef4	Natural	Plant: *Medicago sativa*	Activity against of *Staphylococcus aureus*, *Staphylococcus epidermidis.*
Kalata B1	Natural	Plant: *Oldenlandia affinis*	Activity against *Staphylococcus aureus.*
L-GL13K	Modification	Human: modification *Homo sapiens* salivary protein BPIFA2	Effectively eliminated the infection caused by *Pseudomonas aeruginosa*, *Escherichia coli* and *Streptococcus gordonii* bacteria and reduced the resulting *Pseudomonas aeruginosa* biofilm.

Table 1. *Cont.*

AMP	Origin	Natural Species and the Species on Which the Modified and the Synthetic Peptides Were Based	Activity and/or Effect
D-GL13K	Modification	Human: modification *Homo sapiens* salivary protein BPIFA2	Greater activity against gram-positive bacteria *(Enterococcus faecalis*, *Streptococcus gordoni).*
AG-30/5C	Modification	Human: replacing five residues of *Homo sapiens* peptide AG-30 with five cationic amino acids	Antimicrobial and angiogenic activity (when tested in vivo) in a diabetic mouse wound healing model with methicillin-resistant *Staphylococcus aureus* (MRSA).
P9NaI(SS)	Synthetic	Human: with properties similar to the cationic antimicrobial peptides (CAMP) found in *Homo sapiens*	Greater activity against gram-positive bacteria (*Bacillus subtilis spizenii*, *Staphylococcus aureus*, methicyllin-resistant *Staphylococcus aureus*–MRSA).
DRGN-1	Synthetic	Animal: a derivative of the VK25 peptide found in the plasma of *Varanus komodoensis*	Display potent antimicrobial and anti-biofilm activity; reduction in *Pseudomonas aeruginosa* and *Staphylococcus aureus* species; stimulating keratinocyte migration in a wound closure assay.
Pep19-2.5 (Aspidasept®)	Synthetic	Animal: originally based on the LPS-binding domain of the *Limulus polyphemus* anti-LPS factor (LALF)	Activity against for gram-negative and gram-positive bacteria; promising option for the treatment of acute and chronic wounds most commonly infected with *Staphylococcus aureus* and *Pseudomonas aeruginosa.*
Pexiganan (MSI-78)	Synthetic	Animal: analogue to magainins, which are natural isolated from *Xenopus laevis*	In vitro antibacterial activity of gram-positive and gram-negative, anaerobic and aerobic bacteria.

The immune system of healthy people produces AMPs, but people with diabetes are not immunocompetent due to common venous insufficiency and other DM-related conditions that favor the formation of bacterial biofilms. Human AMPs that are expressed as the body's immune response to injury include defensins (hBD), LL-37 cathelicidin, and dermicidins [69]. For example, RTD-1 is effective against *Escherichia coli*, and human α-defensin HD5 is effective against *Salmonella typhimurium* and *Staphylococcus aureus* infections [65]. One of the most widely used peptides for wounds with impaired healing and infection is LL-37 (cathelicidin), which promotes angiogenesis and re-epithelialization. However, some studies showed that topically applied LL-37 was unstable and was degraded by proteases present in the wound. The possibility of introducing modifications to increase the concentration of endogenous LL-37 in the wounds was therefore investigated. The addition of 1,25-dihydroxyvitamin D3 and L-isoleucine increased the production of hBD-2 and LL-37 during the regeneration process in primary cell cultures from diabetic foot ulcer (DFU) sites [75]. On the other hand, nanoparticle lipid carriers (NLC) encapsulating LL-37 and administered by the local route accelerated wound closure, reconstructed the epithelium and reduced inflammation in vitro and in vivo. Another example is the use of gold nanoparticles conjugated with LL-37. They showed increased wound-healing activity in vivo compared to LL-37 alone due to improved cell migration mediated by EGFR and ERK1/2 phosphorylation [76]. In 2014, LL-37 was introduced into clinical trials for the treatment of venous leg ulcers. In the meantime, another peptide with similar angiogenic properties to LL-37 was developed-the AG-30 peptide. To improve the peptide, five residues of the original sequence were replaced with cationic amino acids. AG-30/5C showed improved antimicrobial and angiogenic activity when tested in vivo in a diabetic mouse wound healing model with methicillin-resistant *Staphylococcus aureus* (MRSA) [69].

Many AMPs with wound healing properties come from amphibians. In many species of the Amphibia family, this process takes less than 10 h (salamander), while in mammals, it can take up to 3 days. The CW49 peptide isolated from frog *Odorrana grahami* skin during diabetic foot syndrome studies promoted angiogenesis and prevented excessive anti-

inflammatory response [65]. Other AMPs isolated from the skin of the red *Rana temporaria* frog (temporin A and B) not only promote wound healing but also kill *Staphylococcus aureus* bacterial cells. Temporin B, the most active of both peptides, kills approximately 80% of bacterial cells at the highest non-cytotoxic dose (16 μM) within 2 h [68]. Other animal AMPs are produced by horseshoe crabs such as a tachyplesin that has a strong biocidal activity against resistant strains of bacteria (*Escherichia coli*, *Staphylococcus aureus*) and fungi (*Candida neoformans*). AMPs are also isolated from plants. MsDef1 and MtDef4 are isolated from *Medicago sativa* inhibit the growth of *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Helicobacter pylori*. In turn, calata B1 and circulina-A show activity against Staphylococcus aureus [65].

However, in order to improve the structural stability and antimicrobial activity, synthetic AMPs have been created. The synthetic SHAP1 peptide shows a strong antibacterial effect, and in the presence of NaCl, it also closes wounds. SHAP1 showed stronger in vitro wound healing activity than LL-37 [77]. Another synthetic peptide is Pep19-2.5, which has antimicrobial activity against Gram-negative and Gram-positive bacteria in skin infections. Pep19-2.5 is believed to be a promising treatment option for acute and chronic wounds, most commonly infected with *Staphylococcus aureus* and *Pseudomonas aeruginosa* [78].

In addition to the abovementioned possibilities, AMPs can be used during combination therapies or in dressings. Combination therapies consist of combining one AMP with another or combining AMP with other antimicrobial agents. Their main advantage lies in the possibility of reducing the development of drug resistance or reducing the occurrence of possible side effects, though some combinations may show a high level of cytotoxicity to mammalian cells [66].

The peptide nisin was tested in combination with pexiganan, all of which was supplied by biogel. This combination has demonstrated the possibility of reducing the concentration of pexiganan required to inhibit and eliminate biofilms formed by isolates from diabetic foot infections, in particular of staphylococcal origin [79].

So far, collagen-based wound dressings (including those for diabetic feet) have been studied as an alternative to dry, conventional dressings. However, as collagen itself does not have antimicrobial protection properties, it was decided to combine collagen with other biocompatible molecules [69]. For example, when a hydrogel containing nanodefensin (NDEFgel) with antimicrobial and immunomodulatory properties was applied topically to a wound surface, it accelerated the wound regeneration process and increased the expression of myofibroblasts and GTP binding protein Rac1 [80]. Another dressing consisting of alginate (ALG), hyaluronic acid (HA) and collagen (COL) was recently used to chemically cross-link AMP Tet213. In vitro drug release studies showed that there was a burst of Tet213 release from ALG/HA/COL-AMP dressings during the first day of incubation, followed by sustained release of the peptide for 14 days. When tested in a rat model of mixed wound infection using *Escherichia coli*/*Staphylococcus aureus*, it was shown that in wounds, the ALG/HA/COL-Tet213 dressing accelerated wound closure and healing and decreased the number of *E. coli* and *S. aureus* bacterial cells compared to the control dressing (ALG/HA/COL + gauze) [81].

Compared to most conventional antibiotics and other methods of treating diabetic wounds, AMPs are a promising alternative as they induce all kinds of beneficial effects.

6. Role of AMPs in Tuberculosis-Diabetes

Pulmonary tuberculosis (PTB) manifests itself as one of the most dangerous contagious diseases in the world, being responsible for 1.8 million deaths annually, according to the World Health Organization [82]. Disturbingly, the cause of death is one organism–*Mycobacterium tuberculosis* [83]. Drugs such as streptomycin, isoniazid (INH), pyrazinamide (PZA), ethambutol (EMB) and rifampin (RIF) are quite successful, but their overuse leads to drug resistance. The number of occurrences of antibiotic-resistant strains is estimated at around 5% [84]. The occurrence of patients with T2D and those with PTB is often geographically similar, and this can lead to a huge health burden on governments [85].

DM is a civilization disease, as stated above, and its incidence is constantly increasing. DM patients are more prone to developing PTB, and combined PTB with DM complicates treatment, increasing chances of relapse and mortality.

MTB is an obligatory pathogen with a small degree of mutation. There is also no data about horizontal gene transfer occurrence [86,87]. MTB is an infectious aerobic microorganism. MTB is a resistant microorganism with a thick waxy coating on a cell surface. Because of this, MTB is well protected both from the outside and inside the host organism. Lungs are the most common target of MTB, accounting for over 85% of cases [88]. One can become infected with MTB when coming into contact with sick people through air droplets. Most of the bacteria are retained in the upper tract and excreted from the body by the mucosal ciliates–only part of them reach the alveoli [82]. From the lungs, the disease can spread through the circulatory system or lymphatic system. Infection in the lungs occurs through contact with the alveoli–inflammatory reactions lead to the development of primary lesions and, as a result, pulmonary fibrosis and destruction of the lungs [88]. MTB enters the body's immune system cells–macrophages and other phagocytic cells–upon contact with lung tissues. It happens via complement receptors, mannose receptors or the type A scavenger receptor. A complex immune response is activated by infection, which allows pathogen molecules to maintain long-term resistance in the host organism [89–91]. Approximately 10% of cases are latent, asymptomatic infections, and dormancy can last many years. A patient's weakened immune system, such as the result of prolonged stress or other diseases such as HIV infection, may lead to activation and acceleration of the infection process. This is an important factor explaining the possibility of MTB survival for many years in the host organism [88]. One of the reasons behind MTB resistance is the structure of its cell wall. It is a complex formation of macromolecules such as peptidoglycans, arabinogalactans and mycolic acids (MAgP complex). Together with proteins and polysaccharides, they create a highly difficult barrier for antimicrobial molecules [82,92]. During infection, the characteristics and arrangement of the components may change [93].

The world is interested in AMPs in the context of TB treatment because of their multifunctional model of activity, natural origin and high efficiency at low concentrations [94]. AMPs are the key players in fighting MTB infection. First is the direct elimination of bacteria through damage to the cell membrane. It occurs as a result of the phospholipid bilayer interruption or the creation of transition pores [82]. The connection between anionic surface elements of mycobacterium cells with cationic AMPs elements promotes permeabilization of the cell membrane [95]. Some AMPs interact with MTB surface proteins, disrupting ion transfer and thus inhibiting the growth and development of bacteria. Their interactions with ATPase inhibit the maintenance of cell pH homeostasis [96]. Although AMPs act mainly on the surface of the cell membrane, some of them can cross it. Synthetic antimicrobial peptides (SAMPs) have a selective antimycobacterial effect. Some SAMPs can penetrate cells and fuse with DNA, thereby inhibiting replication and transcription processes. The effectiveness of this process is achieved at low concentrations of peptides, which reduces potential toxicity to host cells [97]. Bacterial bacilli focus on macrophages–they interfere with their maturation by blocking the transfer of phagocytized complexes to lysosomes [98]. They block the activation of ATPase with a proton pump and the expression of markers necessary to initiate endocytosis. They inhibit the action of phosphatidylinositol kinases and reduce phosphatidylinositol triphosphate (PIP3) levels [99]. This allows the bacteria to survive in the host organism by stopping their own degradation. Hence, many AMPs that promote the formation of phagolysosomes contribute to the fight against tuberculous mycobacteria in the body. After the bacteria penetrate into the macrophages through the appropriate receptors, leukocytes are activated. For effective disposal of the pathogen, the action of endogenous AMPs is necessary. At the initial stage of infection, AMPs can kill bacteria directly. At a later stage, they do this indirectly by modulating the secretion of pro- and anti-inflammatory cytokines [100]. Interestingly, some AMPs showing pro-inflammatory properties in the early stages of infection may be antagonistic in the later

stages [101]. The amount of AMPs present in patients with TB varies depending on the occurrence of DM, the stage of treatment or the stage of the disease.

Cathelicidins are responsible for pro- and anti-inflammatory activities, chemoattractant activity and inducing chemokine expression. These all translate into anti-infective effects. They also exhibit proangiogenic and proapoptotic activity [100]. Macrophages, under the influence of vitamin D and stimulation of toll-like receptor 2 (TLR2) receptors, increase the expression of cathelicidins, which translates into antimycobacterial activity [102]. Patients with TB have higher levels of cathelicidins than uninfected patients. In patients with TB and DM, cathelicidin levels are significantly elevated compared to patients with TB without DM, or patients with DM without TB. The severity of TB is correlated with increased AMP levels [103].

HBD2 is expressed in epidermal cells and produced by monocytes, macrophages and dendritic cells. Its expression is usually induced by pro-inflammatory cytokines [104]. HBD2 expression is induced in various cells with mycobacterial infections [105]. Patients with TB have higher levels of HBD2 than uninfected patients. In patients with TB and DM, HBD2 levels are significantly elevated compared to patients with TB without DM or patients with DM without TB. Increased levels of HBD2 are associated with pathology and disease staging, hence they can serve as biomarkers of disease staging [103].

HNP1, HNP2 and HNP3 belong to the class of defensins. Neutrophils, monocytes, lymphocytes and NK cells are responsible for their production [106]. Their levels are elevated in patients with tuberculosis, regardless of whether they have DM or not. Their number, however, is in no way correlated with the stage of the disease [103].

Granulysin is a protein present in CD8+ T cells that shows antibacterial activity against Mycobacterium TB in vivo and in vitro. However, granulysin levels are lower in TB patients, both diabetic and non-diabetic [103].

It turns out that the amount of AMP in patients changes under the influence of the use of TB drugs in patients. Kumar and colleagues [103] showed that the levels of cathelicidins and HBD2 are decreased in patients after treatment compared to the pre-treatment stage. The level of HNP1-3 and granulysin are increased slightly. This means that effective treatment of TB leads to the restoration of optimal AMP levels in patients with PTB and PTB-DM [103].

7. Phagemids as an Alternative to Antibiotic Therapy

The spread of antibiotic resistance around the world poses a public health threat, so alternatives to antibiotic therapy are being sought. One option might be bacteriophages, which are viruses that attack bacteria. However, despite their numerous advantages, bacteriophages have some limitations. These are, among others, problems with the creation and stabilization of pharmaceutical preparations, the lack of specific activity for a given bacterial strain, reduced activity due to the immune system response or the emergence of bacterial resistance to bacteriophages [107]. So, work began on a modular system of bacterial phagemids. They are modified M13 bacteriophages whose genomes contain plasmid fragments with cloned sequences. Phagemids are smaller than phages but can hold larger pieces of foreign DNA. They are also more efficient during transformation, and there are many restriction enzyme recognition sites in their genome that are convenient for the recombination of DNA and gene manipulation [108]. Phagemids can deliver high copy number plasmids to target cells in one round of infection [109].

The phagemid contains a plasmid with the viral gene encoding the fusion coat protein, the phage origin of replication, and the phage packaging signal. The genes required for phage assembly are provided by the unpackaged "helper" phage. After the phagemid and helper phage co-infect bacteria, proteins are synthesized and assembled around the phagemid DNA [110]. After the infection of the target cell, genes encoding AMP are expressed, which results in inhibition of the vital functions of bacteria [68]. The modular nature of this system allows individual components or the entire network to be modified to target specific bacteria [109].

The effectiveness of phagemids in *Escherichia coli* infection has already been demonstrated [67]. In the case of bacterial infections in people with DM, for example, in diabetic foot syndrome, phagemids encoding AMP may prove to be an alternative to the treatment of ulcers and to the spread of antibiotic resistance.

8. Conclusions

DM is one of the most widespread civilization diseases in the world. Due to its common occurrence and serious, negative health effects of this disease, it is extremely important to conduct research that allows for a more accurate understanding of the mechanisms of this disease and allows the development of new methods of treatment. This type of research includes, among others, research on AMPs and their possible therapeutic role in DM. AMPs can be produced by many types of cells, both immune cells and gastrointestinal cells. They play an important role in fighting many infections that appear in a wide variety of tissues. Current research suggests that AMPs can be used in the treatment of DM. It appears that in both T1D and T2D, AMPs can play a significant role in the prevention and treatment of this disease. Furthermore, in the case of diabetic foot, tuberculosis-diabetes, or diabetic wounds, a positive effect of AMPs was noted. For T1D, three AMPs are currently being researched, CRAMP, CAMP and mBD14. Several types of AMPs associated with this disease have been detected in tuberculosis-diabetes. These include granulysin, HNP1, HNP2, HNP3, HBD2 and cathelicidins. The role of AMPs in this disease is to effectively remove pathogens that cause tuberculosis. In the initial stage of infection, AMPs are directly responsible for killing bacteria. In later stages, it does so indirectly by modulating the secretion of anti-inflammatory and pro-inflammatory cytokines. Currently, several AMPs are known to be involved in controlling the course of DM. At the moment, research is carried out mostly on animal models. Most of these studies are innovative, so more research is still needed for AMPs to be fully introduced into the treatment of DM. New research may result in the discovery of new possibilities of using AMPs in the treatment of diseases such as DM and confirm the therapeutic effect of already known AMPs.

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