Antimicrobial Peptides as Antifungal Agents

Abstract

Antifungal drug resistance has increased greatly causing an alarming shortage in new and effective antifungal reagents. Antimicrobial peptides (AMPs) are present in all living organisms; thus, research into and manipulation of AMPS have influenced the development of many antimicrobial agents including novel antifungals. Antimicrobial peptides play an important role in the first line of defense of the immune system. Natural and synthetic AMPS can stimulate both the adaptive and innate immune system. The development of AMPs as antifungal agents ensures full functionality and target capability depending on certain commonalities such as structure and properties, such as length, size, stability, mechanism of action, and side chains. Recent advancements in research have increased the understanding of AMPs and fungi interaction. This knowledge has prompted new designs and methods of drug development as antifungals for improved patient outcomes. Antimicrobial peptides are important for ongoing clinical trials and have enormous capability as future antifungal agents, giving greater specificity, potency and mode of action against fungal pathogens. The following review will highlight the unique features and mechanisms of antimicrobial peptides that aid in the destruction of fungal cells, as well as identify potential peptides for development as novel antifungal agents and therapies

Antimicrobial Peptides as Antifungal Agents

Fungal infections have been increasing exponentially in worldwide settings. The mortality rates of fungal infections in the developing world are up to 50% while some fungal pathogens mortality rates are at 100% (Van der Weerden, Bleackley, & Anderson, 2013). There are a few antifungal agents, such as azole compounds, used to treat fungal infections. However, the high use of azoles has decreased its ability to treat fungal infections (Wang et al. 2014). The increased use of azole antifungals has lowered the efficacy of these drugs and increased toxic side effects. An alternative to antifungal drugs are antimicrobial peptides (AMPs). AMPs can control fungal infections by targeting the cell membrane then lysing it leading to cell death (Lau, et al., 2015).

Currently, greater than 5,000 AMPs have been discovered or synthesized (Bahar, Ren, 2013). Antimicrobial peptides are oligopeptides composed of 5 to 50 amino acids with shared features such as "[positive] charge, hydrophobicity, and amphipathicity" (Lakshmaiah, & Chen, 2015). AMPSs can be synthesized or found naturally and are the first line of defense against pathogens in prokaryotes and eukaryotes. They are an important part of the innate and adaptive immune system due to their ability to stop infections and reduce inflammatory responses. AMPs, also known as host defensins, exhibit a wide range of activity against bacteria, viruses, plants, fungi and some parasites (Jaskiewicz et al., 2016). "Peptides hBD-2, hBD-3, HNP-1, HNP-2, HNP-3, and LL-37 induce chemotaxis of monocytes, neutrophils, eosinophils, immature dendritic, T-lymphocytes, and CD45RO+ CD4+ T cells. LL-37 and the thrombin-derived host defense peptide GKY25, present anti-inflammatory properties as they bind to LPS and consequently attenuate PAMP-mediated immune activation" (De la Fuente-Núñez, Silva, Lu & Franco, 2017).

The action of AMPs depends on its ability to interact with the cell wall or membrane. Antifungal peptides can kill fungi by targeting either the cell wall or intracellular components. AMPs that target the cell wall kill the cells by increasing permeability or forming pores in the cell membrane causing death (Bahar, & Ren, 2013). The secondary structure of the AMPs such as α and β helices help anchor it to the fungus. Synthetic β-sheets express potent antifungal activity. AMP cell wall activity, such as binding to chitin, is important in the peptides function (Lakshmaiah Narayana, & Chen, 2015). In this case the fungal cell death is caused by binding of the fungal cell wall to the AMP structure. The binding manipulates the fungal cell wall into disorder, stress and finally cell lysis (Bahar, & Ren, 2013).

Antimicrobial peptides have both advantages and disadvantages. AMPs are composed of amino acids thus making them easy to modify and restructure. Fully synthetic AMPs can also be constructed by employing recombinant expression or chemical synthesis (Bahar, & Ren, 2013). Synthetic peptides are useful in modifying and designing new AMPs based on the target. AMP protease activity may be stabilized through these modifications. Two major disadvantages of AMPs is their cytotoxicity to humans, and they can be easily degraded by extreme pH and proteases (Patel, & Akhtar, 2017). The AMP has a difficult time folding and may have reduced activity with extreme temperatures, pH and large composition. Large size and composition also impact production costs. Long peptides increase the cost of manufacturing compared to shorter peptides (Bahar, & Ren, 2013). New models and modes of actions are currently being researched for continuation into clinical trials.

Fungal drug resistance has increased resulting in the development of AMPs as a new approach for the possible treatment of fungal infections. AMPs are a significant prospective source of antifungal drugs. The modification of AMPs potency, specificity and mode of action

have demonstrated great efficacy against fungal infections. AMPs continued research and ongoing clinical trials could lead to the development of new antifungal drugs for human administration.

Literature Review

The increased frequency of fungal infections has raised the need for new antifungal drugs. Antimicrobial peptides are a potential new source of antifungal agents (Panja, Majee, Bandyopadhyay & Maity, 2016). In a recent study by Panja et al. (2016), antifungal peptide composition, structure and physiological properties of full length peptide were analyzed to determine their stability. Analysis of their results revealed the composition of antifungal peptides amino acids. These amino acids contain nonpolar residues making them naturally hydrophobic, while "their domain contains polar charged and uncharged residues illustrating its hydrophilic character" (Panja et al., 2016). The hydrophobicity and compactness increase in the presence of non-polar amino acids on the side chains of the antifungal proteins. The antifungal peptides' rigidity and interactions are maintained by the beta helices formed by the peptides hydrophobic pockets. The degree of interaction with the fungal peptides is increased by the polar charged functional domains of the amino acid residues (Panja et al., 2016). In addition, they found cystine residues, that suggest the presence of disulphide bridges. These bridges stabilize the domain area of the peptide and the beta helices. The active structure of hydrophilic residues cystine, lysine and arginine are important in the interaction of the cell wall of the fungus as well. Interaction of the fungal peptide with the fungus also increases with high random strand coiling, thus making the proteins more flexible. Fungal activity may be inhibited by the flexibility of the protein. Flexibility of the antifungal domain increases in the presence of polar cystine and lysine in addition with nonpolar glycine, alanine, and leucine (Panja et al., 2016). These structural and

physiological characteristics are significant to the design and process of future models of AMPs from a variety of organisms to act as potent antifungal agents.

Because of their unique features, researchers have been able to design AMPs while lowering the manufacturing costs. In a 2015 study, Lau et al. strived to identify and develop topical agents from 30 ultra-short peptides provided by a previous study. Researchers conducted an experiment to compare antimicrobial activity of ultra-short AMPs on common skin pathogens, including *Candida albicans*, for the development of topical drug agents (Lau et al., 2015). Antimicrobial activity was measured by the minimum inhibitory concentration (MIC), including nystatin, a current antifungal drug used as a control to compare MIC results. MICs less than or equal to 6.25μM were found to be potent antifungal agents. Peptides with fewer than eight residues lacked potent antimicrobial activity (Lau et al., 2015). The study revealed several potent antifungal peptides. Two octapeptides with a +5 charge were twice as potent with a MIC of 1.56 μM against *Candida albicans* compared to nystatin with a MIC 3.125μM. Another octapeptide and nonapeptide exhibited antifungal activity with MICs of 6.25 μM. Lau et al. (2015) also revealed that these short peptides of approximately eight residues, including the amino acids isoleucine and arginine, significantly lowered the manufacturing cost. Cationic residues, arginine and lysine, helped determine antifungal activity (Lau et al., 2015). Lower manufacturing costs of potent antifungals are crucial for the development of new and effective antifungal drugs.

AMPs have a broad range of abilities for fighting many organisms. Antimicrobial peptides play a significant role in the innate and adaptive immune system. AMPs wide range of antimicrobial activity and immunomodulatory functionalities protect from many pathogenic infections (De la Fuente-Núñez et al., 2017). As mentioned previously, AMPs are also known as

host defensins. These important immunomodulators stimulate the adaptive immune response. When fungi interact with lectin receptors, T-helper17 (Th17) lymphocytes are stimulated and act to destroy fungi by inducing inflammatory responses and cytokine release. These Th17 lymphocytes then stimulate production of host AMPs (Armstrong-James et al., 2017). AMPs acting as immunomodulatory agents enhance or suppress phagocytosis activity because they are the main therapeutic targets. Understanding these mechanisms of action guide researchers in the development of new drugs and therapies for fungal infections. Many of these agents are currently in clinical trials. However, only certain fungi, *Candida*, *Aspergillus*, *Cryptococcus*, and *Pneumocystis*, can be destroyed because there is lack of understanding of the rare pathogenic fungi (Armstrong-James et al., 2017). Using AMPs as immunomodulatory agents to destroy pathogenic fungi generates even greater future drug development potential.

Discussion

Recently, the increase in drug resistance to antifungals has initiated the discovery of the usefulness of manufactured antimicrobial peptides as potent antifungal agents. Every living organism possesses AMPs which stimulate the adaptive and innate immune system. Certain AMP characteristics determine antifungal activity. Antimicrobial peptides have both hydrophobic and hydrophilic activity. AMPs hydrophobicity from the nonpolar residues glycine, alanine, leucine and isoleucine, increase the peptide solidity (Panja et al., 2016). These nonpolar residues decrease protease activity and cell toxicity. The hydrophobicity of the peptides allows β-sheets to form and have stronger interactions with the fungal cell wall (Bahar, & Ren, 2013). Hydrophilic cystine residues found on the domain of the antifungal peptide are important in stabilizing the beta helices and the cell wall interactions. The β-sheets bind onto the fungal membrane increasing permeability, causing distress until fungal cell lysis occurs (Bahar, & Ren,

2013). New peptides to be used as antifungal agents can thus be developed with these unique characteristics to induce fungal cell death more efficiently than the currently prescribed drugs. Based on this research, antimicrobial specificity and mode of action ensure greater efficacy of the AMPs killing activity, which certainly warrants future use of AMPs for fungal treatments.

Long chains of AMPs have limited drug production capability due to high manufacturing costs (Lau et al., 2015). The understanding of several unique features of AMPs has guided researchers to develop new short and highly potent AMPs against pathogenic fungi. In recent studies, four of thirty ultra-short peptides developed were found to have strong antimicrobial activity against *Candida albicans*. Peptides with less than eight residues and MICs less than 6.25μM did not provide efficient antifungal activity. Several octapeptides and a nonapeptide displayed strong antifungal activity. Two specific octapeptides had stronger potency, MICs of 1.56 μM, than the nonapeptide and other octapeptide with MICs of 6.25 μM. The peptides cationic activity with the residues arginine and lysine helped express antifungal activity. The reduction of amino acids on the AMP structure improved cell specificity and human cell toxicity. Moreover, shorter AMPs lowered the manufacturing cost, aiding in the development of these four potent peptides as antifungal drugs (Lau et al., 2015). These AMPs have demonstrated great efficacy against fungal infections, yet more clinical trials are needed for FDA approval.

Natural and synthetic AMPs can be constructed to have lower specificity and thus lower toxicity to humans and can also be induced to treat human fungal infections. Antimicrobial peptides work in both the innate and adaptive arms of the host immune system to stop infections and regulate inflammatory responses. Since AMPs are also known as host defensins, these antimicrobial peptides are induced as immunomodulatory agents (De la Fuente-Núñez et al., 2017). Immunomodulators stimulate the adaptive immune system by inducing pro-inflammatory

signals. AMPs are produced when Th17 cells are stimulated by the interaction of the fungus with pathogen-associated molecular patterns (PAMPs) (Armstrong-James et al., 2017). PAMPs are molecules present on the cell surface of pathogens which stimulate the innate system to destroy infectious pathogens (Abbas, Lichtman, & Pillai, 2015). These PAMPs promote the peptides to induce chemotaxis on myeloid and lymphoid cell lines. AMPs also help enhance and suppress phagocytic activity. Understanding this immunomodulatory activity is guiding the development of synthetic AMPs for novel therapies and drugs, so as to overcome the increasing antifungal resistance observed in many pathogenic fungi.

Largely, AMPs are great alternative as antimicrobials for fungal infections. However, there are still many limitations on their ability to treat different fungal pathogens. Currently, only the four main common fungal pathogens, *Candida*, *Aspergillus*, *Cryptococcus*, and *Pneumocystis*, are being tested for antifungal agents (Armstrong-James et al., 2017). Additional, pathogenic fungi have not yet been fully tested for treatment. AMPs have potent properties as antifungal agents and could be seen in the future for human administration as prospective antifungal treatments.

Conclusion

Antifungal drugs are not as effective as they used to be. AMPs play a role in both the adaptive and innate immune system in the eradication of fungal infections. AMPs act as immunomodulators by stimulating the adaptive immune response by the secretion of defensins Every organism possesses AMPs, which are important in the immune system. Antimicrobial peptides have become a new source of antifungal agents. The improved understanding of the antimicrobial composition and structure has led to the development of more efficient AMPs in overcoming current resistance by killing fungal infections through greater specificity and

potency. Antimicrobial peptides have been reconstructed with shorter peptides to be less toxic and more cost efficient to produce. By controlling and redesigning AMP structure, antimicrobial peptides have become shorter, with reduced toxicity and greater specificity. Overall, antimicrobial peptide regulation has influenced fungal treatment by lowering manufacturing cost for further human administration and clinical trials. The alarming reduced efficacy, and therefore shortage, of current antifungal agents has initiated the development and modification of AMPs as novel antifungal agents that have greater specificity, potency and mode of action. With continued advancements in research and technology, the use of AMPs as treatment for pathogenic fungal infections is highly possible in the near future.

References

Abbas, K. Abul., Lichtman, H. H. Andrew., Pillai, Shiv. (2015). Basic Immunology: Functions and Disorders of the Immune System. San Francisco, CA. Elsevier Health Sciences

Armstrong-James, D., Brown, G. D., Netea, M. G., Zelante, T., Gresnigt, M. S., van de Veerdonk, F. L., et al. (2017). *Immunotherapeutic approaches to treatment of fungal diseases* doi:https://proxy.lccc.wy.edu:2207/10.1016/S1473-3099(17)30442-5

Bahar, A. A., & Ren, D. (2013). Antimicrobial peptides. *Pharmaceuticals, 6*(12), 1543-1575. doi:http://proxy.lccc.wy.edu:2145/10.3390/ph6121543

De la Fuente-Núñez, C., Silva, O. N., Lu, T. K., & Franco, O. L. (2017). Antimicrobial peptides: Role in human disease and potential as immunotherapies doi:https://proxy.lccc.wy.edu:2207/10.1016/j.pharmthera.2017.04.002

Jaskiewicz, M., Orlowska, M., Olizarowicz, G., Migon, D., Grzywacz, D., & Kamysz, W. (2016). Rapid screening of antimicrobial synthetic peptides. *International Journal of Peptide Research and Therapeutics*, 22(2), 155-161. doi:http://proxy.lccc.wy.edu:2145/10.1007/s10989-015-9494-4

Lakshmaiah Narayana, J., & Chen, J. (2015). Antimicrobial peptides: Possible anti-infective agents. *Peptides*, 70 (2015), 88-94. doi:https://proxy.lccc.wy.edu:2207/10.1016/j.peptides.2015.05.012

Lau, Q. Y., Choo, X. Y., Lim, Z. X., Kong, X. N., Ng, F. M., Ang, M. J., . . . Chia, C. S. Brian. (2015). A head-to-head comparison of the antimicrobial activities of 30 ultra-short antimicrobial peptides against staphylococcus aureus, pseudomonas aeruginosa and candida albicans. *International Journal of Peptide Research and Therapeutics, 21(1), 21-28.* doi:http://proxy.lccc.wy.edu:2145/10.1007/s10989-014-9440-x

Panja, A. S., Majee, S., Bandyopadhyay, B., & Maity, S. (2016). Antifungal signature: Physicochemical and structural in silico analysis of some antifungal peptides. *International Journal of Peptide Research and Therapeutics*, 22(2), 163-169. doi:http://proxy.lccc.wy.edu:2145/10.1007/s10989-015-9493-5

Patel, S., & Akhtar, N. (2017). Antimicrobial peptides (AMPs): The quintessential 'offense and defense' molecules are more than antimicrobials. *Biomedicine & Pharmaceuticals, 95(2017), 1276-12-83*. doi:https://proxy.lccc.wy.edu:2207/10.1016/j.biopha.2017.09.042

Van der Weerden, N.,L., Bleackley, M. R., & Anderson, M. A. (2013). Properties and mechanisms of action of naturally occurring antifungal peptides. *Cellular and Molecular Life Sciences, 70*(19), 3545-70. doi:http://proxy.lccc.wy.edu:2145/10.1007/s00018-013-1260-1

Wang, K., Yan, J., Dang, W., Xie, J., Yan, B., Yan, W., . . . Wang, Rui. (2014). Dual antifungal properties of cationic antimicrobial peptides polybia-MPI: Membrane integrity disruption and inhibition of biofilm formation. *Peptides, 56 (2014), 22–29*. doi:https://proxy.lccc.wy.edu:2207/10.1016/j.peptides.2014.03.005