Antifungal peptides: Origin, activity, and therapeutic potential

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Summary

Antifungal peptides have been identified in a wide range of life forms which include plants, mammals, and microorganisms. Their structures are as varied as their antifungal properties. Semisynthetic and fully synthetic analogs have been developed from a few of these natural peptides that are superior to the parent compound. A few of these peptides hold promise in combating fungal infections and have entered clinical trials.

Key words Antifungal, Peptides, Mycoses

Péptidos antifúngicos: origen, actividad y potencial terapéutico

Resumen

Se han identificado péptidos antifúngicos en una amplia gama de seres vivos, incluyendo plantas, mamíferos y microorganismos, cuya estructutura es tan variada como sus propiedades antifúngicas. A partir de algunos de éstos péptidos naturales, se han desarrollado análogos sintéticos o semisintéticos cuya actividad es superior a la del compuesto original. Unos pocos de éstos péptidos parecen prometedores en la lucha contra las micosis y se han iniciado con ellos ensayos clínicos.

Palabras clave Antifúngico, Péptidos, Micosis

The antifungal properties of peptides have been studied for nearly forty years. During the past 10-15 years, interest in their antifungal nature has expanded due to increased resistance of fungal pathogens to currently-employed antifungal drugs and the toxicity or adverse host reactions of other antiinfectives [1]. Approximately 100 peptides have been investigated to date for their antifungal properties. They vary widely in source with the most studied being natural though an increasing number are semisynthetic or totally synthetic. They are linear or cyclic structures with hydrophobic or amphipathic properties. Activity may be by lysis [2], by binding to, and disruption of, the outer membrane. Others penetrate the membrane and interact with specific internal targets [3,4] or cause pore formation resulting in leakage of important intracellular contents [5]. This review will briefly discuss peptides with moderate-to-excellent activity against fungal pathogens, especially those now undergoing clinical trials.

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Bacterial peptides

Among the first antifungal peptides isolated were the iturin and bacillomycin families produced by *Bacillus subtilis* [6,7]. Both have cyclic peptidolipid structures [8,9] and though antifungal, are hemolytic [10]. Strains of *Pseudomonas syringae* produce the syringomycins, syringostatins, and the syringotoxins, which have potent antifungal properties. Syringomycins are small lipodepsipeptides and are among the most potent bacterial antifungal peptides yet discovered. Syringomycin-E (SE) the most prevalent form of this peptide. SE was highly lethal to *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Fusarium moniliforme,* and *Fusarium oxysporum*, producing an LD95 of 7.8 µg/ml for *A. flavus* and LD95 values with 1.9 µg/ml for the other fungi [11,12]. A 12% ointment of SE was effective in controlling vaginal candidiasis in a murine model [13]. Though generally considered a phytotoxin, SE is produced by a saprophytic isolate from wheat [14], thus casting doubt to its phytotoxic reputation and possible harm to mammals. Syringostatin A and syringotoxin B were lethal to human pathogens such as *Candida albicans* (MIC 3.2 µg/ml) and *A. fumigatus* (MIC, 5 µg/ml) [15]. Cepacidines are glycopeptides from *Burkholderia cepacia* and are noteworthy in that, when used together, were more active than amphotericin B and active against a wide range of fungi including *Candida* sp., *A. niger, Cryptococcus neoformans,* and *F. oxysporum* [16,17]. The nikkomycins, non-toxic to mammalian cells, are a family of potent antifungal peptidyl nucleoside antifungals produced by *Streptomyces tendae* which inhi-

bit chitin biosynthesis [18,19]. Nikkomycins X and Z produced minimum inhibitory concentrations (MICs) of only 0.77 and 0.1 µg/ml, respectively, against *Coccidiodes immitis* with higher MICs of 8 and 30 µg/ml, respectively, against *Blastomyces dermatitidis* [20]. They were very effective in murine models of coccidiomycosis and blastomycosis and well tolerated orally, with moderate efficacy against histoplasmosis [20,21]. Taken orally, nikkomycins X and Z prevented death in mice infected with a lethal challenge of *C. immitis*. However, Nikkomycin Z was degraded in rat, mouse, and rabbit plasma much faster than in pH 7.5 buffer [22].

Fungal peptides

As a group, fungal-produced antifungal peptides are more active than those from bacteria and plants. Echinocandins are a diverse group of potent antifungal peptides which affect cell-wall biosynthesis [23, 24]. They include the echinocandin family (of which echinocandin B is the most common form) and other natural products having a modified echinocandin B peptide core such as the pneumocandins, aculeacins, WF11899, and mulundocandins. Several excellent reviews of this group have been published [25-27]. The native echinocandin family are cyclic lipopeptides produced by *A. nidulans* and *Aspergillus rugulosus* [28, 29]. Echinocandin B is a potent antifungal with a MIC of 0.6 µg/ml for *C. albicans* [28]. Pneumocandins, produced by *Zalerion arboicola,* were effective against *Pneumocystis carinii* in rats [30,31]. *Aspergillus aculeatus* produces aculeacin, a lipopeptide effective against *C. albicans* (MIC 0.2 µg/ml) but not against filamentous fungi [32-34]. Mulundocandins, lipopeptides produced by *Aspergillus syndowi* var. *mulundensis,* are active against *A. niger* with a MIC of 31.3 µg/ml [35]. WF11899 A, B, and C are produced by *Coleophoma empetri*. While more effective than cilofungin and fluconazole in mice against a systemic *Candida* infection, the WF11899 family were hemolytic [36,37]

While native echinocandins and pneumocandins are potent antifungals, they are hemolytic and have poor solubility in water. Much research has been performed to enhance their fungicidal properties while reducing their negative aspects. A series of echinocandin analogs in which substitutions at the N-acyl side chain improved their potency, spectrum of activity, and safety profile have been developed. LY121019 (cilofungin), the first echinocandin introduced into clinical trials, had antifungal activity essentially limited to *Candida* spp. [38], but clinical trials were abandoned due to side affects attributed to its vehicle [39,40] and development was discontinued.

A second generation of molecules belonging to the echinocandin group has now entered clinical trials and consists of VER-002 (LY303366), FK463, and caspofungin (MK-0991). These molecules have been modified to permit solubility in aqueous solution and have potent antifungal activity against *Candida* spp. and *Aspergillus* spp.

V-echinocandin (LY303366) is characterized by a substitution of the cyclic peptide ring. This compound has potent *in vitro* activity against experimental disseminated candidiasis and esophageal candidiasis in profoundly immunocompromised animals [41-44]. V-echinocandin also improves survival and reduces organism-mediated tissue injury in experimental pulmonary aspergillosis [45]. A recently completed clinical trial found V-echinocandin to be highly effective in treatment of esophageal candidiasis in the higher dosage arm [46].

FK463 has a distinctive sulfonate substitution on the peptide ring which enhances its aqueous solublity [47]. This molecule has potent activity *in vivo* against experimental disseminated candidiasis as well as encouraging experimental activity against disseminated aspergillosis [48]. Current clinical trials studying FK463 include a randomized trial of prophylaxis in stem-cell transplant recipients, and open label studies of salvage therapy for invasive aspergillosis and candidiasis. A phase I study for safety, tolerance, and plasma pharmacokinetics in children has recently been completed [49] while a phase I clinical study in healthy adult males determined that FR463 was well tolerated at single infusion concentrations of 2.5-25 mg [50]. Phase II clinical trials showed FR463 effective in improving or clearing clinical symptoms of esophageal candidiasis on AIDS patients [51].

A-192411.29 is a novel antifungal agent derived from natural echinocandin [52]. Its potency was comparable to that of amphotericin B and had broad-spectrum fungicidal activity and was active against clinically important yeasts such as *C. albicans, Candida tropicalis* and *Candida glabrata* [52].

The semisynthetic pneumocandin, L-693,989, is a phosphate ester of pneumocandin A. It had a 90% minimum effective dose of 0.15 mg/kg of body weight and a 90% minimum effective dose of 3.0 mg/kg in animal models of *P. carinii* pneumonia (PCP) and candidiasis, respectively [53]. L-731,373 and L-733,560 are water-soluble, semisynthetic daughter compounds of pneumocandin B_o. They are significantly more potent than their parent compound and were relatively non-hemolytic as compared to amphotericin B [54,55]. They were also effective against disseminated aspergillosis and candidiasis but not cryptococcosis in murine models and delayed mortality due to pulmonary aspergillosis at the effective dose of 5 mg/kg in the rat [56,57].

A second generation pneumocandin, caspofungin (MK-0991) is characterized by a long akyl N-acyl substitution and is active *in vivo* against experimental disseminated candidiasis and disseminated aspergillosis [58, 59]. Recently completed clinical trials demonstrated excellent responses in patients with esophageal candidiasis and encouraging activity in patients with invasive aspergillosis refractory to, or intolerant of, conventional antifungal therapy [60]. It is currently undergoing clinical investigation in randomized trials for empirical antifungal properties and for treatment of candidemia. A phase I study of the safety, tolerance, and plasma pharmacokinetics of caspofungin for early empirical antifungal therapy in children is being initiated.

The aureobasidins, produced by *Aureobasidium pullulans*, are believed to be lytic by altering actin assembly and delocalizing chitin in fungal walls, although another study indicated an effect on sphingolipid synthesis [61-63]. Aureobasidin A has a broader spectrum of activity and a greater effectiveness for murine candidiasis than the echinocandins, fluconazole and amphotericin B [64,65]. Other families of potent antifungal peptides include the leucinostatins and helioferins. Unfortunately, they were toxic to mammalian cells *in vitro*, mice, chicken embryos, or were hemolytic [66,67].

Plant peptides

Though the largest number of antifungal peptides have been isolated from plants, few have been tested against human pathogenic fungi, and even fewer were effective at low concentrations. Zeamatin, a large peptide of 22KDa produced by *Zea mays*, is one such peptide, with a MIC for *C. albicans* of 0.5 µg/ml [68]. It permiabilizes fungal membranes, causing death. Several plant pep-

tides were shown by zonal inhibition studies using treated discs to be active against fungal pathogens. The cyclopeptide alkaloids amphibine H, frangufoline, nummularine, and rugosanine A were active against *A. niger* at 5 µg/ml in such studies [69,70].

Insect and amphibian peptides

Insects and amphibians exist in microorganism-rich environments, so it is not surprising they produce potent antimicrobial peptides. Several insect antifungal peptide families have been discovered, with the cecropins being the most well known. Cecropin A and B are linear, lytic peptides produced by the giant silk moth, *Hyalopora cecropia*, and are lethal for approximately 95% of *F. oxysporum* and *A. fumigatus* germinating conidia at 12 and 9.5 µg/ml, respectively [71,72]. Interestingly, both cecropins were active in acidic medium (pH 5-6) but only cecropin A was fungicidal at neutral pH. This may be due to a charge difference at the C-terminus (the peptide portion that inserts into the membrane) of these peptides [72]. Recently, an all D-amino acid-containing cecropin B was found to retain the potent fungicidal properties of the L-form, but resistant to degradation by papain, trypsin and pepsin which destroyed the L-form [73]. Drosomycin and thanatin are cysteine-rich peptides from *Drosophila melanogaster* and *Podisus maculiveris*, respectively. Drosomycin is an inducible peptide, 44 amino acids in length, with a twisted three-stranded -sheet stabilized by three disulphide bonds and is very effective against *F. oxysporum* isolates [74-76]. Thanatin is smaller than drosomycin, being only 21-residues in size, and, in water, adopts a well-defined antiparallel -sheet structure with a disulfide bridge [77]. It is non-hemolytic with activity against *F. oxysporum* and *A. fumigatus* [78].

Three families of amphibian antifungal peptides have been isolated. The dermaseptins, produced by the South American arboreal frog, *Phyllomedusa sauvagii* are lytic, linear, cationic, lysine-rich peptides [79-81]. Dermaseptin was fungicidal for *A. flavus, A. fumigatus,* and *F. oxysporum*, with LD50 values of 3 µM, 0.5 µM, and 0.8 µM, respectively [72]. Another South American tree frog, *Phyllomedusa bicolor*, produces Skin-PYY (SPYY), an antifungal compound closely related to NPY, a neuropeptide, and PYY, a gastrointestinal tract peptide. SPYY permeates phospholipid membranes and inhibited *C. neoformans, C. albicans,* and *A. fumigatus* growth with MIC values of 20 µg/ml, 15 µg/ml, and 80 µg/ml, respectively [82]. Magainins, the first reported antifungal amphibian peptides, are produced by the African clawed frog, *Xenopus laevis*. Their helical, amphiphilic structures have an affinity for microbial membranes causing dissipation of ion gradients [83, 84]. Magainin was not hemolytic and inhibited *C. albicans* growth [83].

Mammalian antifungal peptides

Mammals produce potent, lytic antimicrobial peptides. Excellent reviews [85-90] have been published on this topic. Antifungal peptides produced by neutrophils and intestinal Paneth's cells are known as α-defensins while β-defensins are mainly produced by epithelial cells. Human neutrophils produce HNP-1 and HNP-2 which were lethal for *C. albicans* while they and HNP-3 significantly inhibited *C. neoformans* growth at 50 µg/ml [91,92]. Rabbit neutrophils produce NP-1, NP-2, and NP-3a, which were highly effective against *C. albicans* [93]. NP-1 inhibited the growth of encapsulated *C. neoformans* isolates between 3.75 and 15 µg/ml, and prevented the growth of nonencapsulated isolates at lower concentrations [94]. NP-1,-2, and -3 killed 100% of *A. fumigatus* hyphae at 25, 50, and 100 µg/ml, respectively, but were inactive against the resting conidia of this fungus [95].

β-defensins include tracheal antimicrobial peptide (TAP) and the gallinacins. The cysteine-rich TAP is produced by bovine respiratory epithelial cells and was active (MIC of 25 µg/ml) against *C. albicans* [96]. Chicken leukocytes produced the cationic gallinacins which have three intramolecular cysteine disulfide bonds and are lysine-and arginine-rich [97]. Gallinacin-1 and -1α inhibited *C. albicans* growth at 25 µg/ml [97]. β-defensins also include the porcine leukocyte produced cationic and cysteine-rich protegrins. Protegrins-1,-2, and -3 inhibited *C. albicans* growth at 60, 8, and 3 µg/ml, respectively [98,99].

Bactericidal/permeability-increasing protein (BPI) is a 55 kDa basic protein present in the azurophilic granules of polymorphonuclear leukocytes. The fungicidal properties reside in Domain III (amino acids 142-169) of this protein. A series of analogs (called XMP peptides) based on the BPI molecule have a broad spectrum of activity, some with low MIC values against pathogenic fungi [100]. For example, XMP peptides produced the following MIC values for pathogenic fungi: *C. neoformans* (0.31-1.25 µg/ml), *F. solani* (0.31-10 µg/ml), and *C. krusei* (1.25-5 µg/ml).

Summary

Nature has developed many defense mechanisms to protect life against fungal infections. Among them are the antimicrobial peptides produced by diverse life forms. Over 100 natural peptides or their analogs have been found with varying activities against pathogenic fungi though, to date, only a few have entered clinical trials. Undoubtedly, many more remain to be discovered and, because analogs can be more potent than their parents, future research will certainly find novel antifungal peptides with potential pharmaceutical utility.

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