Human Mycoses: Drugs and Targets for Emerging Pathogens

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Fungal infections in humans range from superficial and cutaneous (such as dermatophytoses) to deeply invasive and disseminated (such as candidiasis and cryptococcosis). Pathogenic fungi occur worldwide, although particular species may predominate in certain geographic areas.

In the past 20 years, fungal infections have increased dramatically—paradoxically, as a result of medical advances. Fungal infections occur more frequently in people whose immune system is suppressed (because of organ transplantation, cancer chemotherapy, or the human immunodeficiency virus), who have been treated with broad-spectrum antibacterial agents, or who have been subject to invasive procedures (catheters and prosthetic devices, for example) (1). Fungal infections are now important causes of morbidity and mortality of hospitalized patients: The frequency of invasive candidiasis has increased tenfold to become the fourth most common blood culture isolate (2). Invasive pulmonary aspergillosis is a leading cause of mortality in bone-marrow transplant recipients (2), while *Pneumocystis carinii* pneumonia is the cause of death in many patients with acquired immunodeficiency syndrome in North America and Europe (3). Many opportunistic fungal infections cannot be diagnosed by usual blood culture and must be treated empirically in severely immunocompromised patients (4).

Treatment of deeply invasive fungal infections has lagged behind bacterial chemotherapy. There are substantially fewer antifungal than antibacterial drugs. Amphotericin B, still the "gold standard" for the treatment of invasive fungal infections, was discovered in the late 1950s. Why this apparent neglect? First, like mammalian cells,

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fungi are eukaryotes and thus agents that inhibit fungal protein, RNA, or DNA biosynthesis are likely to do the same in the patient, producing toxic side effects. Second, life-threatening fungal infections were thought, until recently, to be too infrequent to warrant aggressive research by the pharmaceutical industry. In the past decade, however, many more antifungal drugs have become available. Nevertheless, there are still major weaknesses in their spectra, potency, safety, and pharmacokinetic properties.
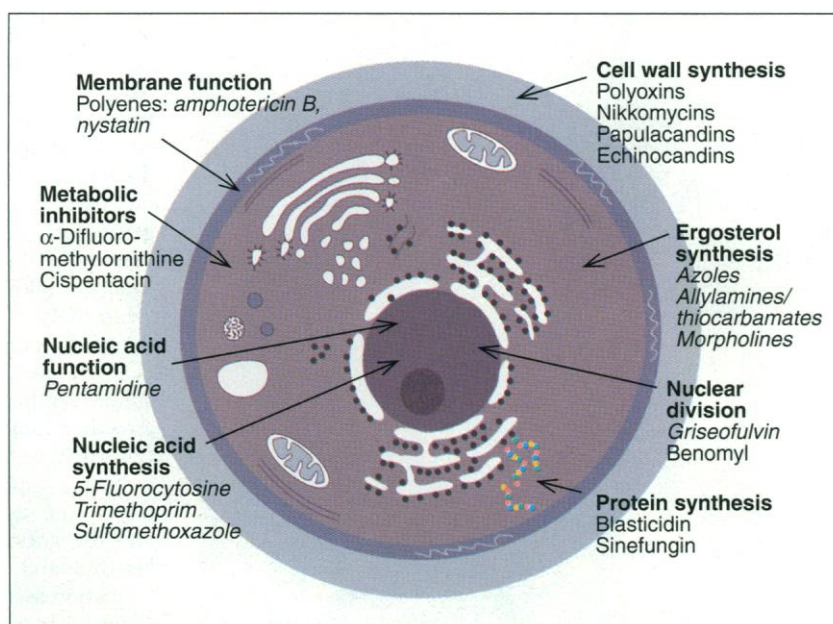

Fig. 1. Sites of action of some antifungals (26). Compounds used clinically are italicized.

Three major groups of antifungal compounds are in clinical use: the polyene antibiotics, the azole derivatives, and the allylamines-thiocarbamates (Fig. 1). All interact with or inhibit ergosterol, the major sterol in the fungal plasma membrane. [These drugs are thus ineffective against *P. carinii*, which has cholesterol instead of ergosterol (5) in its membrane.]

The polyene antibiotics, produced by *Streptomyces* species, are fungicidal and act against more types of fungi than other antifungals (6). They bind to membrane sterols, causing increased membrane permeability, leakage of cytoplasmic contents, and cell death. The clinically useful polyenes—amphotericin B, nystatin, and pimaricin—have a higher affinity for ergosterol than its mammalian counterpart, cholesterol, thus limiting their toxicity in

mammalian cells; nephrotoxicity is the major side effect. Amphotericin B, the only polyene used in invasive mycoses, is particularly appropriate for the treatment of deeply invasive mycoses in severely immunocompromised patients, in spite of its considerable acute and chronic toxicity. The serious side effects may be reduced by new delivery systems, such as liposomes, lipid complexes, and colloidal dispersions (7).

The azole derivatives are completely synthetic, and many new compounds in this class have become available (8). In general, the broad antifungal activity of azoles includes most yeasts and filamentous fungi. Depending on whether the azole has two or three nitrogens in the five-membered azole ring, it is classified as an imidazole or triazole. The azoles act primarily on ergosterol biosynthesis at the C-14 demethylation step, a cytochrome P-450–dependent reaction (Fig. 2). The resulting ergosterol depletion and accumulation of methylated sterols interferes with the "bulk" functions of ergosterol: The structure of the plasma membrane is altered, making it more vulnerable to further damage and altering the activity of several membrane-bound enzymes, such as those for nutrient transport and chitin synthesis. Severe depletion of ergosterol may also interfere with the hormone-like ("sparking") functions of ergosterol and affect cell growth and proliferation (9). The overall effect is fungistatic rather than fungicidal, limiting the utility of these drugs. Nevertheless, azoles (ketoconazole, fluconazole, and itraconazole) are being used more frequently in the treatment of systemic mycoses, even in severely immunosuppressed patients. The older imidazoles also interfere with ergosterol functions but, in addition, can interact with and damage the cell membrane directly; they are consequently fungicidal and toxic and therefore used only topically (8). The newer antifungal azoles do not produce serious toxicity; however, they influence the endocrine system by inhibiting mammalian cytochrome P-450–dependent enzymes that synthesize steroid hormones. These effects are more pronounced with imidazoles than with triazoles; ketoconazole, for instance, inhibits testosterone synthesis at 100 times lower concentration than fluconazole (10).

There are two allylamine antifungals in

clinical use, naftifine (topical) and terbinafine (oral), and one thiocarbamate, tolnaftate (topical) (11). All three inhibit squalene epoxidase which, together with oxidosqualene cyclase, cyclizes squalene to lanosterol (Fig. 2). The resulting ergosterol depletion and squalene accumulation affect membrane structure and functions such as nutrient uptake. Squalene epoxidase is not a cytochrome P-450 enzyme, and thus this class of compounds is less toxic than the azoles. Because of their poor pharmacokinetics, these compounds work only against dermatophytes, although squalene epoxidase is ubiquitous and allylamines have broad antifungal activity in vitro.

Less commonly used drugs include the morpholines: For example, amorolfine, which is used in the topical treatment of nail infections, also acts on the ergosterol pathway (12). Morpholines inhibit two steps in the ergosterol pathway, Δ^{14} reductase and Δ^7-Δ^8 isomerase (Fig. 2). Other targets in the ergosterol pathway are oxidosqualene cyclase and Δ^{24} methyltransferase (13), the latter being unique to ergosterol synthesis and thus a particularly attractive target. However, even targets with counterparts in the cholesterol pathway may be clinically useful, because contrary to fungi, which have no uptake system for sterols, mammalian cells can take up dietary cholesterol via the low density lipoprotein (LDL) pathway (14). Targets in the early steps of ergosterol synthesis include hydroxymethyl glutaryl coenzyme A (CoA) and mevalonic acid synthesis (13), although so far inhibitors of these steps do not have antifungal activity—probably because they cannot penetrate the membrane.

The fluoropyrimidine 5-fluorocytosine (5-FC) has a limited activity spectrum and is mainly used in combination with amphotericin B in cryptococcal meningitis (15). It is deaminated to 5-fluorouracil (5-FU), converted to the nucleoside triphosphate, and incorporated into RNA where it causes miscoding. In addition, 5-FU is converted to deoxynucleoside, which inhibits thymidylate synthase and thereby DNA biosynthesis.

The fungal cell wall, a structure essential to fungi that has functions similar to its bacterial counterpart and that is lacking in mammalian cells, would seem to be an ideal target for antifungal agents. Inhibitors of the biosynthesis of two important cell wall components, glucan and chitin, already exist. Polyoxins and the structurally related nikkomycins (both consist of a pyrimidine nucleoside linked to a peptide moiety) inhibit chitin synthase competitively, presumably acting as analogs of the substrate uridine diphosphate (UDP)–N-acetylglucosamine (chitin is an N-acetylglucosamine homopolymer), causing inhibition of septation and osmotic lysis (16). Unfortunately, the target of polyoxins and nikkomycins is in the inner leaflet of the plasma membrane; they are taken up by a dipeptide permease, and thus peptides in body fluids antagonize their transport. Appropriate polyoxin derivatives that bypass peptide transport have been synthesized (16). Another hopeful development is the observed synergy between chitin synthase inhibitors and glucan or ergosterol synthesis inhibitors (17), although its clinical significance is not

Fig. 2. Sites of action of some clinically useful ergosterol synthesis inhibitors.

yet known. Papulacandins and the cyclic lipopeptides echinocandins inhibit $\beta(1,3)$ glucan synthase and are being studied with increasing intensity (18). Echinocandin derivatives have promising in vivo fungicidal activity, including activity against P. carinii (19).

Topoisomerase I and II control the topological state of DNA so that it can undergo replication, transcription, repair, and chromosomal segregation (20). The success of topoisomerase inhibitors in antibacterial and anticancer chemotherapy has made fungal topoisomerases particularly attractive drug targets (20). However, it is not yet known whether fungal topoisomerases have exploitable differences relative to their mammalian counterparts.

Elongation factor 3 (EF-3), a 125-kD protein, participates in fungal but not mammalian protein synthesis (21) and is another promising target. It is present in most fungi and is essential for viability—disruption of its gene is lethal (21). It is specifically required by the yeast 40S ribosomal subunit, although its exact function in the elongation cycle is unclear. A major drawback for rational drug design is the absence of known EF-3 inhibitors.

The success of the antibacterial agents trimethoprim and sulfomethoxazole in treating and P. carinii pneumonia has validated their sites of action in the folate pathway as drug targets for this organism, although not for other fungi. Toxicity considerations would preclude DNA itself as being an antifungal target, although it is the likely target of the antipneumocystis drug pentamidine (22). After the discovery of cispentacin which inhibits homoserine dehydrogenase (23), the possibility of interfering with amino acid synthesis emerged, although available information may not yet be sufficient to launch a synthetic, rational drug design program. Ornithine decarboxylase (ODC), the rate-limiting enzyme in polyamine synthesis and a favorite target for growth inhibition in cancer chemotherapy (24), may be another antifungal target. Microtubule aggregation—the target for griseofulvin, the agricultural fungicide benomyl, and the anticancer drugs vincristine, vinblastine, and taxol (25)—also merits reexamination as a possible target for clinical antifungals. More speculative means to combat fungi include inhibiting protease activity, decreasing adhesion through alternate ligands, and preventing phase transition to the more invasive form, or preventing virulence in general, through effects on signal transduction.

The recent surge in the use of antifungal agents, particularly azoles, is shifting the population of fungal pathogens toward species that are intrinsically resistant (for example, non-albicans Candida species) and is selecting resistant strains of susceptible species. Despite recent progress, problems in detection and susceptibility testing continue to affect treatment. Permeability constraints, far less rigorously studied in fungi than in bacteria, have prevented potent inhibitors of promising targets from becoming potent antifungals. New approaches and chemical entities are urgently needed, since the conditions that led to the emergence of fungal infections in the first place are likely to persist in the future.
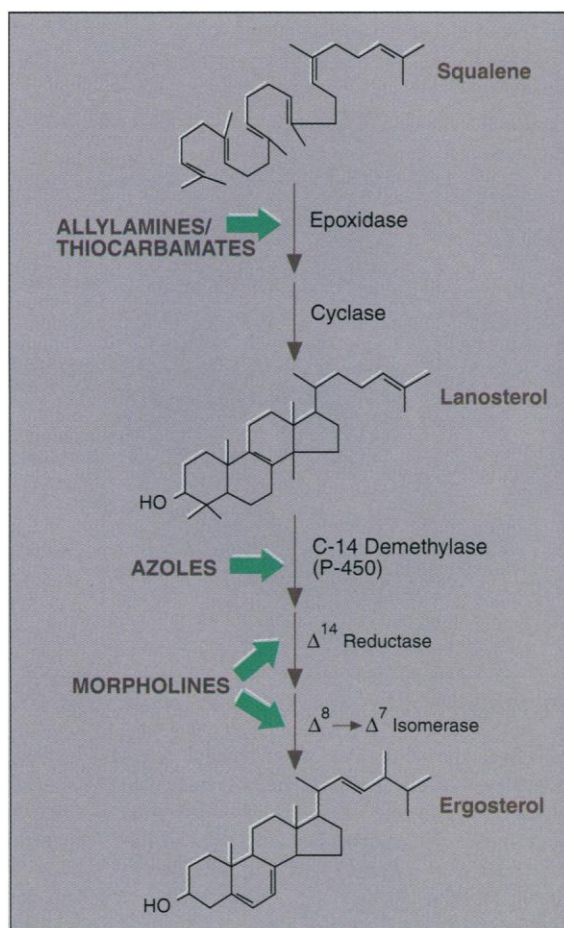
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26. We thank D. Pappagianis for providing the basis for the figure.

Ubiquitous Natural Antibiotics

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Bacteria and fungi coexist with all animals and plants in close physical association. Indeed, health depends on the existence of natural flora that provides vitamins and stimulates the development of specific immune responses in higher vertebrates. However, when particular bacteria or fungi are introduced into a host in a niche that permits their growth as well as the elaboration of virulence factors, disease can result. Given the number and diversity of organisms with which we are in constant contact, this result is thankfully rare—likely because of our defense systems. One such system is that of nonspecific immunity, which can proceed along either oxidative or nonoxidative antimicrobial pathways.

These nonoxidative defense pathways comprise a wide variety of oligopeptides and proteins with potent antibacterial and antifungal activity. Such proteins occur in all animals and plants in which they have been sought. A recent meeting sponsored by the Ciba Foundation (18 to 20 January 1994) brought together scientists working on these antimicrobial proteins. Despite the wide variety of sources and identities of these peptides, a number of common themes emerged.

On the basis of sequence and structural information, these antimicrobial agents can be arranged in groups, as indicated in the table. The low molecular weight (3000 to 5000) peptides are processed from larger precursors. Some are linear molecules and some contain highly conserved disulfide bonds. In addition, there are families within these two groups. Among the linear molecules, the cecropins, which have been isolated from both insects and mammals (*1, 2*), and the magainins (*3*) form well-defined groups on the basis of highly conserved sequence similarities. A specific motif characterized by proline and arginine occurs in the bactenecins Bac-5 and Bac-7 (*4*) and in cecropin PR-39 (*2*) from pig intestine. This motif may be important for the mechanism of action of these peptides, as discussed below.

The disulfide-containing peptides include three families, defined on the basis of the number of disulfide bonds as well as sequence similarity. Defensins are a common, highly conserved group of peptides (*5*).

With the exception of the serprocidins,

the high molecular weight proteins are less easy to arrange in families. The serprocidins are related by virtue of their structural similarity to other serine proteases (*6*).

These molecules are widespread within the organism. The ability of phagocytes to kill ingested microbes has been recognized since the time of Metchnikoff in the late 19th century; it is from the granules of these cells that antimicrobial proteins were originally identified. More recently antimicrobial proteins have also been found in secretions such as seminal fluid, lymph, and serum. Most striking is the recent discovery that a variety of tissue surfaces either directly expose or possess cells that can exocytose antimicrobial peptides. These surface peptides include the magainins from frog skin and defensin-like peptides derived from both the granulated epithelial cells in the mammalian small intestine and the tracheal mucosa (*7*). Thus, it seems that most locations that are in contact with indigenous microorganisms are equipped to limit their inappropriate multiplication.

In addition to their antimicrobial activity, many antimicrobial proteins have functions that are not directly related to killing microorganisms. The serprocidins cathepsin G and proteinase 3 are proteases for connective tissue matrix components and are probably responsible for tissue damage during inflammation (*6*). Although protease activity per se is not required for microbe killing, many of the antimicrobial peptides are present in locations that are protease-rich. Proteolysis may allow the antimicrobial protein to gain easier access to the site of infection. Proteinase 3 is also a determinant of myeloid differentiation in the human leukemia HL-60 cell line (*6*). The relation among antimicrobial, proteolytic, and developmental activities of this protein is obscure but certainly deserves further attention. Coincidentally, sapecins (insect defensins) have also been implicated as growth factors for fly embryos (*8*). BPI, azurocidin, and the tachyplesins also bind lipopolysaccharide (LPS); this ability may facilitate their interactions with Gram-negative organisms, yet LPS binding alone is insufficient for antimicrobial activity because the major LPS-binding protein from mammalian serum, LBP, has no detectable antimicrobial activity (*6, 9, 10*). In addition to binding to LPS, BPI can neutralize LPS activity and thus must contribute to host defense against endotoxin.

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