Antifungal Drug Resistance

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The increasing incidence of invasive fungal infections is the result of many factors, including an increasing number of patients with severe immunosuppression. Although new drugs have been introduced to combat this problem, the development of resistance to antifungal drugs has become increasingly apparent, especially in patients who require long-term treatment or who are receiving antifungal prophylaxis, and there is growing awareness of shifts of flora to more-resistant species. The frequency, interpretation, and, in particular, mechanism of resistance to current classes of antifungal agents, particularly the azoles (where resistance has climbed most prominently) are discussed in this review.

INCIDENCE OF FUNGAL INFECTIONS

Life expectancy has increased by at least 10 years since the first use of antimicrobial drugs for the treatment of microbial infections [1]. Interestingly, this success has also increased the number of fungal infections. The incidence of fungal infections varies widely according to the underlying condition. In solid organ–transplant recipients, the incidence ranges from 5% after renal transplantation to 35% in lung and heart transplant recipients and up to 40% after liver transplantation [2, 3]. Cancer chemotherapy and allogeneic bone marrow transplantation are associated with fungal disease, and up to 30% of patients with acute leukemia experience invasive fungal infections. Candidemia has increased markedly in hospitalized patients. Mucosal and systemic fungal infections are also common in patients with AIDS. Oral candidiasis (or colonization of the mouth, pharynx, and esophagus) was seen in up to 84% of patients with HIV infection [4, 5], and 10% of patients with AIDS in the pre-HAART era developed cryptococcal meningitis, one of the most prevalent AIDS-defining infections [6]. Histoplasmosis, coccidioidomycosis, and infections with *Penicillium* spp. play important roles in certain geographical regions in both immunocompetent and immunocompromised hosts.

DEFINITION OF RESISTANCE

The term “resistance” is used to describe a relative insensitivity of a microbe to an antimicrobial drug as tested in vitro and compared with other isolates of the same species. In contrast, clinical failure describes failure of an appropriate therapy for a certain indication to result in a clinical response. The reason for clinical failure may be, for example, antifungal resistance, but other causes, such as an impaired immune function, poor bioavailability of the drug given, or an accelerated metabolism of the drug, are possible causes of treatment failure [7].

Primary resistance occurs in organisms never exposed in that host to the drug of interest. In contrast, secondary resistance, also defined as acquired resistance, arises only after exposure of the organism to the drug. Intrinsic resistance is defined as resistance of all or almost all isolates of one species to a certain drug—for example, the resistance of *Candida krusei* to fluconazole. In addition, the term “clinical resistance” can be used to describe failure of therapy or relapse of infection with an organism not associated with in vitro resistance—for example, changes in the host’s immune system, such as neutropenia [7].
ANTIFUNGAL DRUGS

The appropriate antifungal therapy and selection of drug should be based on criteria such as immune status of the host, site of infection, characteristics of the infection (the fungal species and its susceptibility to different antifungal drugs), and pharmacokinetic characteristics of the drug (e.g., absorption, elimination, and toxicity). Only a limited number of antimycotic drugs are available for the treatment of systemic fungal infections. The mode of action of the most important antifungal drugs can be divided into 4 different classes [11], as follows:

1. Polyene macrolides that lead to an alteration of membrane functions (amphotericin B [AmB] and its lipid formulations);
2. Azole derivatives that inhibit the 14a lanosterol demethylase, a key enzyme in ergosterol biosynthesis (ketocanazole, fluconazole, itraconazole, and voriconazole);
3. DNA and RNA synthesis inhibitors (fluconazole); and
4. 1,3-β-glucan synthase inhibitors (echinocandins).

POLYENES

Activity and mechanism of action. AmB is active against a wide variety of fungi, including yeasts and molds such as Candida spp. and Aspergillus spp., Cryptococcus neoformans, Zygosomyces, dimorphic fungi, and some dematiaceous fungi. The target structure is plasma membrane ergosterol, where AmB forms a channel. Through this channel, the fungal cell leaks potassium ions, resulting in a disruption of the proton gradient [12]. It is hypothesized that a ring of 8–10 polyene molecules form aqueous pores within the membrane bilayer structure. In addition, AmB causes oxidative damage to plasma membranes [11, 13]. In higher concentrations, polyenes also inhibit chitin synthase, a cell wall synthetic enzyme localized in the membrane. The interaction between AmB and human cell membranes containing cholesterol results in toxic side effects of the drug. The most important side effect is an impairment of renal function due to decreased filtration in the glomeruli.

Frequency of resistance. Reports of AmB resistance are limited. Primary resistance to AmB exists in some isolates of Candida lusitaniae [14], C. lipolectica [15], and C. guilliermondii [16, 17]. Trichosporon beigeli [18, 19], Pseudallescheria boydii [20], and Scopulariopsis spp. often show intrinsic resistance to AmB. Until now, invasive infections caused by these fungi have been relatively infrequent. Secondary resistance has occasionally been described in Candida spp. as well as in C. neoformans [16, 17, 21–25]. In vitro susceptibility to AmB of Aspergillus species has, in one study, been correlated with the clinical outcome of invasive aspergillosis [26]. Noble et al. [27] reported the isolation of AmB-resistant C. albicans from patients with leukemia. Bryce et al. [28] observed that Candida isolates with MICs of 0.78 μg/mL led to a higher mortality among patients with candidemia. Powderly et al. [17] noted that there is a correlation between failure of AmB therapy and in vitro resistance in yeasts isolated from cancer patients with invasive fungal infection. Lack of activity of AmB is described in systemic murine fusarial infection [29] as well as in clinical isolates [30]. In a study investigating 22 isolates of Fusarium spp., Arikan et al. [31] noted that AmB MICs spanned a relatively narrow range. The MICs were higher for Fusarium isolates than for Aspergillus isolates, with slightly higher MICs for F. oxysporum than for F. solani.

Whether repeated treatment with AmB, or other factors such as treatment with immunosuppressive chemotherapy, is responsible for polyene resistance still needs to be clarified. Patients with cryptococcal meningitis often receive AmB over long time periods [32]. Casadevall et al. [33] tested the susceptibility of C. neoformans isolates from patients with recurrent meningitis to AmB and found no increased MIC values. Reasons for recurrence might have been poor penetration of the drug, inconsistent compliance with other drug regimens, or deterioration of the immune system of the host.

It has been hypothesized that concurrent or previous therapy with azoles might lead to AmB resistance, such as AmB-refractory aspergillosis after itraconazole therapy [34]. The mechanism might be altered sterol type or content in the membrane, the result of the azole therapy. Kelly et al. [35] observed cross-resistance to AmB in 2 fluconazole-resistant C. albicans strains isolated from patients with AIDS. The strains were accumulating 14α-methylfecosterol instead of 14α-methylergosta-8,24-dien-3β,6α-diol, causing the cross-resistance. The same group reported 2 AmB-resistant mutants of Ustilago maydis with cross-resistance to nystatin. An alteration of the ergosterol biosynthesis was precluded as the cause of polyene resistance in these isolates [36]. Resistance to nystatin and AmB of a C. albicans isolate has been also documented in a immunocompetent trauma patient [37].

For Aspergillus terreus, it was shown in a murine model of disseminated aspergillosis that the mortality rate and survival time were identical for mice treated with AmB and untreated mice. This demonstrates the high resistance of this strain to
AmB [38]. Only a few reports exist about the prevalence of resistance to the lipid formulations of AmB. Results of in vitro testing with these formulations may be of limited relevance because the active moiety of the complex in vivo is the amphotericin, which is released from sites of tissue binding of the lipid-amphotericin complex. Moreover, even for that fraction of drug circulating as the complex, tissue lipases may release the active drug in vivo [39]. Treatment with AmB is often guided by toxicity rather than by therapeutic efficacy, whereas lipid formulations have reduced toxicity, thus allowing higher doses to be administered. In neutropenic mice infected with an Aspergillus fumigatus strain with reduced susceptibility to AmB, it was shown that high doses of liposomal AmB or amphotericin lipid complex could overcome reduced susceptibility to the standard preparation, AmB deoxycholate [40]. It is known that lipid formulation alters tissue distribution [41]. Selective increased uptake in certain tissues could produce improved results.

Mechanism of resistance. The cause of resistance to polyenes is a significant alteration of the lipid composition in the plasma membrane (e.g., a reduction in the ergosterol content due to a lack of the Δ^{24} (14)-isomerase) [42, 43]. This circumstance leads to a lower affinity of AmB to the plasma membrane, probably the result of a lack of the binding site [44, 45]. However, for a highly resistant strain of A. terreus, it was shown that the major sterol in its plasma membrane was ergosterol, suggesting that resistance to AmB was not related to a modified sterol composition [38].

Another cause for AmB resistance may be an altered content of β-1,3 glucans in the fungal cell wall. These components, which increase the stability of the cell wall, influence the access of large molecules such as AmB to the plasma membrane [46]. Seo et al. [47] showed that alteration of glucans led to AmB resistance in A. flavus.

ERGOSTEROL BIOSYNTHESIS INHIBITORS

Activity and mechanism of action. In the 1970s, a new class of antifungal drugs was developed, the azoles. The first azole available for systemic use was clotrimazole. However, its use was limited largely because of inconsistent concentrations in the blood. Miconazole was the first effective azole for systemic infections, but its use was limited because it could only be given intravenously and it offered few advantages over amphotericin, aside from lessened toxicity [48].

Ketoconazole was the first azole to be orally available and showed consistent blood levels [49]. Susceptibility to ketoconazole was documented for the majority of Candida species, with the exception of C. glabrata [50, 51]. Ketoconazole was successfully used for the treatment of chronic mucocutaneous candidiasis. Ketoconazole also shows activity against Coccidioides, Histoplasma, Blastomyces, and dermatophytes. In addition to ketoconazole’s rare hepatotoxicity, resistance was described, especially in patients with mucocutaneous candidiasis or AIDS and oropharyngeal and esophageal candidiasis [52–55].

Itraconazole, the first triazole used in humans, is available for oral (capsules and cyclodextrin solution) and intravenous administration. Cycloheximide enhances the bioavailability of the drug [56], and the intravenous preparation offers the possibility of reliably high serum levels, which is especially a concern in patients with impaired absorption. Itraconazole shows broad in vitro activity against Candida spp., but also against Aspergillus spp. and dimorphic and dematiaceous fungi. Fluconazole, another triazole that can be administered orally and intravenously, is used for prophylaxis and treatment of oropharyngeal and esophageal candidiasis in patients with AIDS as well as in neutropenic patients [57, 58]. It could be demonstrated that after chemotherapy or bone marrow transplantation, the incidence of invasive candidiasis could be significantly reduced by using fluconazole prophylaxis [59–61]. In addition, fluconazole was used in nonneutropenic [62, 63] and surgical patients [64] with Candida colonization or invasive candidiasis. Fluconazole has only limited activity against molds and other filamentous fungi. Use of fluconazole in prophylaxis has resulted in a shift of host flora to resistant species and would be expected not to alter (and may even increase) the frequency of infection due to molds.

Voriconazole, structurally related to fluconazole, shows a spectrum similar to itraconazole. It is active against C. krusei, C. guilliermondii, and C. lusitaniae [65]. Walsh et al. [66] recently published a randomized, international, multicenter trial comparing voriconazole with liposomal AmB for empirical antifungal treatment. They conclude that voriconazole is a suitable alternative to AmB preparations for empirical antifungal treatment in patients with neutropenia and persistent fever. In the same issue of the journal, other views, including that of the US Food and Drug Administration [67], as well as an editorial [68] concerning empirical antifungal therapy with voriconazole, were published. Voriconazole was approved in the United States for limited indications in May 2002. Posaconazole and ravuconazole, 2 additional triazoles, are currently under investigation. Posaconazole has in vitro and in vivo activity against Aspergillus [69], Cryptococcus [70], and Coccidioides [71] as well as Candida spp. [72]. Although penetration into CSF is low, there is activity against cerebral phaeohyphomycosis in animals. Ravuconazole (BMS 207147) shows in vitro activity against dimorphic fungi, such as Coccidioides, Histoplasma, and Blastomyces, as well as against Aspergillus, Fusarium, and fluconazole-sensitive and -resistant Candida species (e.g., C. krusei), and against the key dermatophytes [73].

Ergosterol is the most prevalent sterol in the fungal plasma membrane. Azoles act as ergosterol synthesis inhibitors by bind-
ing to lanosterol demethylase, a specific enzyme in ergosterol biosynthesis. The active binding site of lanosterol demethylase contains a heme domain [74, 75]. Azoles bind with a specific nitrogen atom in theazole ring nucleus to the iron atom of the heme domain, preventing the demethylation of lanosterol [76]. Azoles may also target lipids of the fungal plasma membranes [76] and may interact with the 3-ketosteroid reductase, an enzyme in methylsterol biosynthesis.

**Frequency of resistance.** Azole resistance is frequently described in patients with AIDS and mucosal candidiasis, oral candidiasis, or deep-seated candidiasis. Oropharyngeal colonization with *Candida* species was found in as many as 84% of patients infected with HIV, and symptomatic oral disease was found in up to 46% of these patients [4, 5]. Many of these patients require long-term treatment to suppress oropharyngeal and esophageal candidiasis. As a consequence, the number of patients with candidiasis refractory to azole treatment has increased markedly [77–84]. Resistance to azoles has been reported in 32% of symptomatic patients and in 14% of asymptomatic patients [4, 5, 85]. The prevalence correlates with the amount of CD4 cells, the fungal load, and the duration and doses of therapy [86]. Maenza et al. [86] reported that mean CD4 counts were lower (11 vs. 71 cells/mm³) and the duration of azole therapy was longer (419 vs. 118 days) in patients with fluconazole-resistant candidiasis. However, Vuffray et al. [87] showed that resistance can also occur in patients with high single doses of fluconazole. Recently, resistant *C. neoformans* was isolated from an immunocompetent patient without previous exposure to fluconazole [88]. Pelletier et al. [89] documented in HIV-infected children resistance of *C. albicans* to clotrimazole. They demonstrated a significant risk of cross-resistance to other azoles and showed that the resistance correlated with refractory mucosal candidiasis. Besides *C. albicans*, primary resistance has been also reported for *C. dubliniensis*, *C. norvegensis*, and *C. inconspicua* [90–92].

*Candida* species such as *C. krusei* are intrinsically resistant to fluconazole. They have been isolated in high frequency among HIV-positive patients [93], as well as cancer, bone marrow–transplant, and surgical patients [94–96]. There is controversy over the reason for the increasing incidence of intrinsically resistant non-*albicans* Candida species. The cause might be a selection of these species due to the widespread administration of fluconazole [95, 97]. Other causes might also be nosocomial transmission [98] or transmission between partners [99].

**Mechanism of resistance.** Different potential molecular mechanisms of azole resistance are possible. Some of these mechanisms are well known from antibacterial resistance. Modified target enzymes have been well characterized as a mechanism of resistance to penicillin in *Streptococcus pneumoniae* and *Neisseria gonorrhoeae* [100], efflux pumps have been implicated in resistance to chloramphenicol and tetracycline [101], and resistance to aminoglycosides has been associated with alterations in the plasma membrane [102]. Sometimes development of resistance to an azole drug leads to cross-resistance to other azoles, and sometimes the resistance is azole specific [103]; this will depend on the specificity of the resistance mechanism (e.g., the affinity of the target enzyme or the pump for a particular molecular structure). Rustad et al. [104] have recently reported a correlation of azole resistance in *Candida albicans* with homozygosity at the mating-type–like (MTL) locus, suggesting that key resistance genes may be regulated by this locus or in the vicinity of this locus.

### ALTERATION OF DRUG EFFLUX

Alteration of drug efflux has been described as a recognized mechanism of resistance in bacteria (e.g., in *S. pneumoniae* resistance to macrolide antibiotics [105]). Furthermore, ATP-binding cassette transporters have been studied extensively and are known to cause drug resistance. These proteins span the plasma membrane several times and contain 2 ATP-binding cytoplasmic domains [106, 107]. In *Candida, Candida* drug resistance (CDR) genes have been associated with azole resistance. At least 5 different CDR genes (CDR1 to CDR5) have been described. CDR1 is a transporter protein in *Candida* and *Cryptococcus*, for which azoles (and other drugs—e.g., cycloheximide and chloramphenicol) are substrates [108, 109]. Its structure is related to the P-glycoprotein in humans, which is associated with resistance to chemotherapeutic drugs. Sanglard et al. [109] and Albertson et al. [110] compared the ability of *C. albicans* to accumulate fluconazole. In both studies, azole-resistant strains showed reduced intracellular concentrations of fluconazole. Northern blot testing showed as much as a 10-fold increase of CDR1 mRNA, indicating overexpression of CDR1. Parkinson et al. [111] described the accumulation of fluconazole in susceptible and resistant isolates of *C. glabrata*, where reduced ability of the resistant isolate to accumulate fluconazole was a consequence of energy-dependent drug efflux. Sanglard et al. [112] reported that *C. albicans* mutants that carry a CDR1 deletion showed hypersusceptibility to fluconazole, itraconazole, and ketoconazole as well as terbinafine and amorolfine. CDR2 overexpression was also shown to confer resistance to azoles. Strains with *CDR1* and *CDR2* double disruption were more hypersusceptible than strains with a single *CDR1* disruption [113]. In addition, overexpression of MDR1 was observed in fluconazole-resistant *C. albicans* isolates [109, 114].

MDR1 (also known as *BEN*) was originally described to encode resistance to benomyl and methotrexate [115]. This transporter uses membrane potential instead of ATP as the energy source. Sanglard et al. [109] have demonstrated that if
the *Candida MDRI* gene was cloned into *S. cerevisiae*, overexpression of this gene potentially mediated resistance to azoles. Rhodamine 123, a fluorescent compound transported by MDR pumps, was used by Clark et al. [116] to search for the presence of these pumps in *C. albicans* and *C. glabrata*. They found evidence that in azole-resistant fungi, rhodamine 123 accumulated. In *C. glabrata*, a competition between rhodamine 123 and fluconazole for the transport out of the cell was demonstrated. Parkinson et al. [111] found an efflux competition between benomyl and fluconazole. Sanglard et al. [117] demonstrated that an increased expression of an ATP-binding cassette transporter gene plays a determinant role in the acquisition of resistance toazole antifungals in clinical isolates of *C. glabrata*. Recent studies suggest the resistance associated with brief exposures to high-dose azoles is transient (as opposed to stable resistance associated with long-term exposure to low-dose azoles) and associated with alterations in transcriptional regulation of CDR pump expression.

**ALTERATION OF THE TARGET ENZYME**

Overexpression of the target enzyme of the azoles, the lanosterol demethylase, was found to be a mechanism of resistance in *C. glabrata* [118]. This *C. glabrata* strain was resistant to fluconazole and itraconazole. An increased copy number of the lanosterol demethylase may result in increased ergosterol biosynthesis. However, in *C. albicans*, overexpression of the lanosterol demethylase probably accounts only for a minor effect on the development of resistance, because the expression demonstrated was only 3 times higher than in fluconazole-susceptible isolates [109].

As outlined above, azoles act as ergosterol synthesis inhibitors by binding to the lanosterol demethylase. The gene encoding this protein is *ERG11*, also known as *ERG16*, and the enzyme has been referred to as CYP51A1 in *C. albicans*. Alterations of this enzyme caused by defined point mutations in *ERG11* have been described to be associated withazole resistance. White [119] described the presence of the amino acid substitution of lysine for arginine at position 467 (R467K). The substitution is located near the heme cofactor and thus causes structural and/or functional enzyme alterations. A substitution at amino acid 464 (G464S) in azole-resistant *C. albicans* isolates has been described [120]. Kelly et al. [121] confirmed that this mutation, resulting in changes in the heme-binding domain, causes fluconazole resistance through substantially reducing the inhibitory effect of fluconazole and described its association with perturbation of the heme environment. Similarly, this could be also demonstrated for the amino acid substitution T315A in *C. albicans*, where again the lanosterol demethylase activity was reduced 2-fold and the affinity to fluconazole was reduced [122], and for Y132H, a substitution that allows resistant mu-

tants to produce ergosterol and retain fitness [123]. Additionally, in 5 clinical isolates of *C. albicans*, an amino acid substitution at position 105 from phenylalanine to leucine was described, potentially leading to reduced accessibility to the active binding site [120]. Fluconazole enters the active binding site through a channel and mutations therein affect the access of substrates and inhibitors. Marichal et al. [124] demonstrated that point mutations in this gene are clustered in 3 regions. Y132H was the only mutation found in the 2 N-terminal regions to be of importance forazole resistance, whereas in the C-terminal region, 3 mutations were associated with resistance.

**ALTERATION OF THE ERG3 GENES**

Altered sterol Δ(5,6) desaturase is another explanation forazole resistance. In azole-sensitive strains treated with azoles, 14-methyl-3,6-diol accumulates and leads to a fungistatic effect, whereas in sterol Δ(5,6) desaturate mutants (due to mutations in the gene *ERG3*), its precursor, 14-methylfecoosterol, accumulates, which can support growth of the fungal cell. In *S. cerevisiae*, >20 fluconazole-resistant isolates were found to have altered sterol Δ(5,6) desaturase [125]. It has been suggested that *ERG3* mutations alone can cause azole resistance in *S. cerevisiae* [126, 127].

In *C. albicans*, Kelly et al. [35, 128] demonstrated in 2 clinical isolates from patients with AIDS that resistance was caused by defective sterol Δ(5,6) desaturase, leading to an accumulation of 14-methylfecoosterol. A consequence of this mechanism of resistance is that an absence of ergosterol causes cross-resistance toAmB. In addition, defective sterol Δ(5,6) desaturase has been described to be responsible for azole resistance in the plant pathogen *U. maydis* [129].

**ALTERATION OF THE DRUG INFUX**

The sterol composition of the plasma membrane can also affect the influx of drug into the cell. Alterations in the plasma membrane composition affect membrane fluidity and asymmetry, leading to a decreased uptake of drug [130]. It was demonstrated that altered phospholipid and fatty acid composition of the plasma membrane of *C. albicans* may lead to resistance to miconazole [131]. Hitchcock and Whittle [132] noted a larger lipid content and a lower polar-lipid-to-neutral-lipid ratio in an azole-resistant *C. albicans* isolate compared with wild-type strains, potentially causing a decreased permeability of the membrane and thus a reduced penetration of azoles. In a fluconazole-resistant *C. albicans* isolate, a decreased amount of ergosterol and a lower phosphatidylcholine:phosphatidylethanolamine ratio in the plasma membrane, which might be responsible for an altered uptake and thus for a reduced intracellular accumulation of fluconazole, was demon-
strated [133]. In an itraconazole-resistant *C. krusei* isolate, it was indicated that reduced accumulation of drug accounts for resistance rather than drug efflux or modifications in the ergosterol [134].

**FREQUENCY OF MECHANISMS OF RESISTANCE**

Perea et al. [135] studied matched sets of 20 fluconazole-susceptible and -resistant isolates from HIV-infected patients with oropharyngeal candidiasis, presumably treated with azoles. They found overexpression of efflux pumps in 85% of resistant isolates (the frequency of overexpression of CDR genes and the *MDR1* gene was similar), amino acid substitutions in the target enzyme lanosterol demethylase in 58%–65%, and overexpression of the gene that encodes the enzyme in 35%–42%. Multiple mechanisms of resistance were found in 75% of resistant isolates. White et al. [136] addressed this question in a different way. They analyzed 38 random isolates, half resistant and half susceptible, and found overexpression of the CDR genes only in (some) resistant isolates and correlated with resistance. Neither *MDR1*, *FLU1*, or *ERG11* overexpression nor amino acid point mutations correlated with resistance or were found frequently in resistant isolates. The latter study [136] suggests that putative resistance mechanisms found in resistant isolates, except for CDR-encoded pumps, may not be responsible for the resistance and that additional mechanisms of resistance remain to be uncovered. Both studies [135, 136] suggested *CDRI* and *CDR2* may be coregulated, and both reported additional data on cross-resistance to other azoles, in addition to that previously described [103].

**INVESTIGATIONAL TRIAZOLES**

In May 2002, voriconazole was approved in the United States for limited indications. Furthermore, to date, 2 new triazoles, posaconazole (Schering Plough) and ravuconazole (Bristol Myers Squibb), are being investigated. Both show potent activity against a variety of yeast and molds. Data on resistance against itraconazole, posaconazole, ravuconazole, and voriconazole. No resistance to posaconazole was reported [140].

**5-FLUOROCYTOSINE**

*Activity and mode of action.* Fluocytosine (5-FC), a fluorinated pyrimidine, has been used to treat fungal infections since the 1960s. It has excellent penetration into body fluids. 5-FC is principally active against yeasts, including *Candida* spp. and *C. neoformans*. Today, 5-FC is usually administered in combination with other antifungal drugs. 5-FC is taken up into the fungal cell by the enzyme cytosine permease, then modified to 5-fluorouracil and converted to fluorouridine triphosphate, which in turn is incorporated into fungal RNA, causing disruption of protein synthesis [141]. 5-Fluorouracil also is converted to fluorodeoxyuridine monophosphate, which interferes with the enzyme thymidylate synthase. Inhibition of thymidylate synthase subsequently causes disruption of DNA synthesis [142].

*Frequency of resistance.* Primary resistance to 5-FC is not uncommon [143, 144]. 5-FC resistance also occurs in *C. neoformans* [12].

*Mechanism of resistance.* Two mechanisms of resistance have been described. First is a mutational decrease of activity of the cytosine permease or deaminase, leading to a decreased uptake or conversion of the drug. This mechanism is responsible for primary and intrinsic resistance [13]. Second is a loss of activity of uracil phosphoribosyltransferase, an enzyme responsible for conversion of 5-fluorouracil to 5-fluorouridyl acid [141, 145]. Whelan and Kerridge [146] described decreased activity of the uridine monophosphate pyrophosphorylase associated with resistance to 5-FC in *C. albicans*. In 29 clinical isolates of *C. albicans* with resistance to 5-FC, no cytosine permease–deficiency mutants were found. In contrast, for *P.apsilosis*, a 5-FC–resistant strain was described that evolved while the patient received therapy and had <7% of the cytosine deaminase activity compared with the parent susceptible isolate [147].

**INHIBITORS OF GLUCAN SYNTHESIS**

*Activity and mode of action.* The cell wall of fungi contains compounds that are not found elsewhere in nature. Some of
these components may provide selective targets for antifungal drugs without target-associated toxicity in mammalian hosts. The fungal cell wall consists of a multilayer structure composed of glucan, chitin, mannan, and mannanprotein [148, 149].

Three antifungal compounds (caspofungin, FK-463, and VER-002) belonging to the class of echinocandins are currently available for clinical use or are under development [150]. All compounds inhibit the fungus-specific biosynthesis of 1,3-β-D-glucan [151]. They have potent in vitro activity against Candida spp. and some activity against Aspergillus, dimorphic molds, and Pneumocystis carinii [152]. As expected, isolates resistant to antifungal triazoles did not show cross-resistance to the 3 compounds [153].

Treatment of fungi with β-glucan synthase inhibitors causes noncompetitive inhibition of 1,3 β-glucan synthase with secondary effects on other constituents, such as an increase in the chitin content of the cell wall and a reduction in the ergosterol content of the fungal cell membrane [154].

**Frequency of resistance.** For C. dubliniensis, an organism closely related to C. albicans and often associated with oral candidiasis in HIV-infected patients, resistance to fluconazole has been described. It was shown that these resistant isolates were highly susceptible to an echinocandin [155]. Cuenca-Estrella et al. [156] compared the in vitro activity of an echinocandin to that of itraconazole and AmB against fluconazole-resistant isolates of Candida. They showed that the echinocandin was more potent against C. albicans, C. glabrata, C. krusei, and C. tropicalis but less potent in vitro against C. parapsilosis and C. guillermondii. Furthermore, it was shown that the relatively low efficacy of an echinocandin against C. neoformans may result from a reduced activity against the glucan synthase of C. neoformans [157].

**Mechanism of resistance.** Data on echinocandin resistance are limited and are based on laboratory-derived mutants of S. cerevisiae. In this fungus, the β-glucan synthase complex is encoded by 2 genes and regulated by a third. Kurtz and Douglas [158] and Kurtz [159] presumed that in S. cerevisiae, mutations in one of the genes, FKS1, cause resistance to echinocandins by alteration of β-glucan synthase. Echinocandins do not penetrate into the cytoplasm of the fungal cell. Thus, resistance mechanisms described for azoles, such as increased activity of efflux pumps or altered sterol composition of the plasma membrane, seem to be irrelevant. Kurtz and Douglas [158] and Kurtz [159] presumed that resistance to β-glucan synthase inhibitors may be very much alike in both organisms, S. cerevisiae and C. albicans.

**CONCLUSIONS**

Advances in medicine have led to more patients living longer. Commensurate with the growth in patients at risk, the number of patients with severe fungal infections has dramatically increased. Concern regarding the development of resistance to any of the few antifungal drugs available has developed. Although we are able to define certain mechanisms of drug resistance, continued efforts for a deeper understanding of the cellular and molecular mechanisms as well as the clinical components of antifungal resistance will be important [8]. In addition, new diagnostic tools for rapid, sensitive, and specific detection of fungi in clinical material, such as PCR-based techniques, are mandatory. Finally, our knowledge of drug-resistance mechanisms should maximize the utility of current drugs and assist in the development of new antifungal drugs and new treatment strategies.

**References**

15. Walsh TJ, Salkin IF, Dixon DM, Hurd NJ. Clinical, microbiological,


