

Antifungal Chemotherapeutics

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Key Words

Antifungals · Azoles · Echinocandins · Fungal infections · Treatment strategies

Abstract

This review addresses trends in outcome and risk factors for invasive fungal infections, current antifungal agents and new therapeutic strategies. Current prospects for new therapies rest upon caspofungin, the first of a new class of antifungal molecules, the echinocandins, and new extended-spectrum azoles, voriconazole, posaconazole and ravuconazole. Approval by the Food and Drug Administration of the USA and the European Medicine Agency was given in 2001–2002 to voriconazole and caspofungin. Voriconazole clearly demonstrated a decrease in mortality in invasive aspergillosis and fusariosis fungal infections.

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Introduction

With the advent of the acquired immunodeficiency syndrome (AIDS) and advancing medical technology (organ transplantation, radiochemotherapy, dialysis and other procedures), fungal infections are assuming increasing prominence and are associated with considerable mortality.

A 'wonder drug' to prevent or cure all invasive fungal infections (IFI) does not exist. All drugs currently avail-

able have both benefits and drawbacks. Unfortunately, amphotericin B (AmB), the agent with the broadest spectrum of activity, is associated with considerable toxicity. The triazoles, for example fluconazole (FLU) or itraconazole (ITRA), have clearly demonstrated their value in the antifungal field, but have not fulfilled all hopes. A truly safe and effective fungicidal drug does not yet exist.

Current prospects for new therapies therefore depend upon caspofungin, the first of a new class of antifungal molecules, the echinocandins, and new extended-spectrum azoles: voriconazole, posaconazole and ravuconazole. In 2001–2002, approval by the Food and Drug Administration (FDA) and European Medicine Agency (EMA) was given to voriconazole and caspofungin. Voriconazole demonstrated something that was not clearly marked before, a decreased mortality in fatal IFI, e.g. in aspergillosis and fusariosis.

This review addresses trends in outcome and risk factors for IFI, current antifungal agents, and new therapeutic strategies.

Resistance to Antifungal Agents

Candida albicans is usually susceptible to all antifungal agents [1–12]. A recent study on the susceptibility of *C. albicans* to FLU showed 99.7% susceptibility in Europe and 98.1% in the US (more than 15,000 strains tested) [2, 5]. Susceptibility to AmB and nystatin is up to 100%, and susceptibility to ketoconazole and ITRA is

also very high (95–100%) [5]. Development of resistance towards 5-fluorocytosine (5-FC) is rapid, but a majority of *C. albicans* infections are still susceptible to all available drugs. The most recent study of Pfaller and the Mycoses Study Group [13] on more than 1,000 *C. albicans* bloodstream isolates showed no increase in resistance in 1999 in comparison to 1990.

In the last 5 years, two studies (both in cancer patients) have tried to address speciation and antifungal resistance. One was a European prospective study [14] and the other a single-institution-based retrospective candidemia surveillance [9]. This latter US study, involving 140 cases, showed that fungemias from non-*albicans Candida* (NAC) spp. had similar outcomes to *C. albicans* fungemias [9]. In contrast, the larger prospective multicenter study conducted by the European Organization for Research and Treatment of Cancer [14] showed that candidemias due to *C. glabrata* (odds ratio, OR, 2.08), *C. tropicalis* (OR 1.8) and *C. krusei* (OR approximately 1.6) had inferior outcomes in comparison to *C. albicans* (OR 1.00) and *C. parapsilosis* (OR 0.48). Other studies on *C. krusei*, *C. lusitaniae*, *C. glabrata* and *C. parapsilosis* [7–11, 15–18] have investigated comparative virulence and mortality from NAC species. These studies showed higher mortality from NAC in comparison to *C. albicans* [14, 7–11, 15].

The in vivo-in vitro correlations between outcome of fungal infections and susceptibility or resistance of causative fungal isolates have been documented in adult HIV-positive patients [4, 6, 19]. There are still controversies concerning the relationship between in vitro susceptibility of *Candida* spp. and clinical outcomes in non-HIV patient populations [7–13, 15]. One study showed a correlation between minimal lethal concentration (MLC) and minimal fungicidal concentration to AmB and microbiological and clinical failure [3]. A similar correlation was shown with FLU [18]. A partial relationship between clinical course and mortality of candidemia and in vitro susceptibility to FLU was shown in two studies [8, 12], but not observed in another [5]. All published studies on this issue dealt with *Candida* spp. in adults. Studies in children, mainly in neonates with fungemia and in non-HIV individuals with mucosal/skin mycology are expected to address correlations between outcome and resistance. Furthermore, breakpoints (minimal inhibitory concentrations, MICs) for antifungals should be derived from those studies or slightly adapted, if necessary [1–3, 12–14, 18, 19].

'Old' Therapeutic Molecules

Polyenes; Amphotericin B, Amphotericin B-Deoxycholate and Nystatin

Amphotericin B: Activity, Toxicity

AmpB has long been a mainstay of therapy for systemic fungal infections. However, it is associated with many adverse effects, notably a variety of electrolyte abnormalities (including hypokalemia, hypomagnesemia, hypernatremia and metabolic acidosis) and most importantly, acute renal failure [20–25]. It appears that nephrotoxicity is mediated, in part, through a direct toxic effect on renal tubular cells, resulting in acute tubular necrosis [26]. Vasoconstriction is also induced, with both processes reducing glomerular filtration [26–30]. No specific treatments are available to reverse the acute renal failure associated with AmB, although salt loading (when tolerated) has been used with some success as a prophylactic measure [31–33]. This acute renal failure is often dose limiting, is sometimes irreversible, and can prolong hospitalization and increase treatment costs. The reported frequency of acute renal failure associated with AmB has varied widely [25, 29]. A meaningful decrement in renal function has been reported to occur in up to 80% of patients who receive the drug, and is almost inevitable in patients who receive a cumulative dose of >5 g. However, much smaller total doses are currently used. For example, in one recent study of 102 patients in a community hospital, the mean cumulative dose of AmB used was <200 mg, and the maximum dose was 840 mg. In this population only 15% of the patients developed acute renal failure [25]. Considering the frequency of this complication, relatively little data are available regarding the mortality rates associated with acute renal failure from any cause [34, 35], and it remains to be determined whether patient mortality due to renal failure is caused by the underlying disease or results directly from this complication.

Amphotericin B: Resistance

AmB-deoxycholate or conventional AmB has been used since 1951. The drug has fungicidal activity towards most *Candida* isolates and species (MIC <0.1 µg/ml) if the serum concentration exceeds 10–16 times MIC. In isolates with MIC of 0.5–2 µg/ml, AmB will show fungistatic activity. Clinical resistance may be seen in most cases infected with *Candida* strains that demonstrate MIC >0.5 µg/ml. Although AmB shows in vitro activity against most pathogenic fungi, there are notable exceptions. Most *Fusarium* spp., *Pseudallescheria boydii* and

Paecilomyces varioti are resistant both in vitro and clinically (MIC >2.2–8 µg/ml). Also, *Trichosporon* spp. and some strains of *Aspergillus nidulans*, *Aspergillus terreus*, *C. lusitaniae* and *C. rugosa* show secondary resistance to AmB. Resistance is seen in 1–5% of *C. lusitaniae*, *C. guillemondii* and *C. rugosa* cases [3, 8, 12].

Amphotericin B: Applications

Standard indications for intravenous AmB are systemic fungal infections due to the most common fungal organisms (apart from *Trichosporon* spp. and *Ps. boydii*). Empiric therapy of febrile neutropenia is also a very important indication for AmB [8, 17]. Oral AmB was formerly used for prophylaxis, or selective decontamination, but has been replaced with FLU following bone marrow transplantation or in patients with acute leukemia [16]. For cryptococcal meningitis, AmB in combination with 5-FC is indicated. The standard dose of AmB is 0.7–1 mg/kg/day in adults and 1–1.5 mg/kg/day in children. Lipid formulations of AmB have been developed [36–39] (see below) with reduced toxicity facilitating administration of 3–5 mg/kg/day.

Lipid Formulations of Polyenes

Although AmB and nystatin are antifungal agents with the broadest spectra of activity, the toxicity of polyenes may prevent their use at sufficient dosages [33–36]. Clinical failure is common for patients infected with organisms susceptible in vitro because patients are either under-dosed, or are unable to complete the treatment due to kidney failure (acute or chronic) [40, 41]. Three new lipid-based formulations for polyenes have been developed and introduced from 1990 to 1998 in response to these problems [37–39, 42]. These are (i) ambisome, a ‘true’ liposomal formulation of AmB, (ii) Nexstar (AmBisome, Nexstar) AmB in colloidal dispersion (ABCD; Amphocil, Zeneca) and (iii) AmB in lipid complex (ABLC, Abelcet developed by Enzon, Liposome) and currently offered by Enzon. In addition, liposomal nystatin is under investigation (Nyotran).

In several patients, a dosage of 4–10 mg/kg/day of ABLC was used in AmB-refractory mycosis [36], contrasting with a recommended dose for ambisome of 1–3 mg/kg/day and of 2–4 mg/kg/day ABCD (Amphocil). There were no differences in acute toxicity (chills, fever and shivers) which were lowest (according to randomized controlled studies [38]) in ambisome, which is a ‘true liposomal AmB’, and somewhat higher in ABLC [38]. Kidney and liver toxicity was significantly lower for all lipid formulations than in conventional AmB therapy [38].

Specific indications for lipid formulations of AmB are: preexisting renal failure, acute toxicity or chronic nephro- or hepatotoxicity during treatment with AmB and fungal infection that does not respond to treatment with either AmB or FLU.

Recommended indications for the systemic use of lipid formulations are: (a) fungemia caused by species resistant in vitro to AmB and/or FLU (*C. krusei*, *C. lusitaniae*); (b) invasive aspergillosis (IA) when proven or highly suspected (positive bronchoalveolar lavage, antigens or typical CT scan); (c) fusarial and mucor infections (indicated in combination with granulocyte macrophage colony stimulating factor, GM-CSF, therapy and/or surgery), and (d) any IFI not responding to AmB or FLU; lipid formulations of AmB or voriconazole are the drugs of choice for second-line therapy.

Liposomal amphotericins are significantly more expensive than conventional AmB. Evaluation of costs and the risks of AmB toxicity, along with the relative efficacy and costs of other antifungal agents, could help in decision-making about which patients should receive newer, more costly but less nephrotoxic agents as alternatives to AmB [42]. Because of costs, lipid-based formulations are not indicated for empiric therapy of febrile neutropenia and/or prophylaxis of fungal infections in leukemia or bone marrow transplantation [38].

Antimetabolite Drugs: 5-Fluorocytosine

5-FC is the only antimetabolite currently used as a systemic antifungal agent (Ancotil, Roche) which is active in vitro against *Candida* spp., *Cryptococcus neoformans* and a variety of yeasts and molds. This agent is not used in monotherapy but only in combination with AmB (see section on combination antifungal therapies) or FLU [43]. The main indication for 5-FC is treatment of cryptococcal meningitis (in combination with AmB). FC is no longer used in combination with AmB for empiric treatment of febrile neutropenia, since monotherapy with AmB showed sufficient efficacy in empiric therapy in cancer patients and in cases of neutropenic fever not responding to antimicrobials.

Triazole Drugs

Fluconazole

FLU has excellent in vitro activity against *C. albicans* [44]. FLU can also be effective against some NAC species,

including *C. parapsilosis*, *C. tropicalis* and *C. glabrata*, although higher doses may be required [44, 45]. FLU shows excellent penetration into cerebrospinal fluid, and the peroral form has high bioavailability.

FLU is effective against infections at a wide range of body sites and tissues, irrespective of the patient's immune status [46–48]. Indications in adults include vaginal [47, 49], mucosal [48], dermal and systemic candidosis [46, 49–53]. Prophylactic administration of FLU can be useful in patients considered at risk of fungal infections as a consequence of neutropenia following chemotherapy or radiotherapy [54–58]. Experimental evidence [59] and clinical case reports [60] suggest that prophylaxis with FLU may be useful in preventing infections associated with *C. albicans*.

Fluconazole is suitable and effective for use in children [61–69], but higher dosage adjustments should be made. In the elderly, normal adult dose regimens should be used if there is no evidence of renal impairment. In those with renal impairment, no adjustments in single-dose therapy are required; for multiple-dose therapy, either the dosage interval should be increased or the daily dosage should be reduced [46, 69].

Itraconazole

ITRA shows in vitro activity against a greater range of *Candida* species than FLU [70]. However, ITRA does not penetrate into the cerebrospinal and peritoneal fluid, and its bioavailability is poor. ITRA capsules are effective and indicated for the treatment of a number of localized and systemic fungal infections in adults, irrespective of the immune status of the patient [71]. These include vulvovaginal [72] and oropharyngeal candidosis. Because of its lipophilicity, ITRA distributes to the nails, and the capsule formulation is effective in the treatment of onychomycosis [73]. ITRA capsules can be used as maintenance therapy in patients with AIDS and as prophylaxis before expected neutropenia. As absorption is often impaired, blood monitoring should be performed and the dose increased if necessary.

There are inadequate data concerning ITRA capsule formulations, or in solution, in children (<12 years) and in the elderly for its use to be recommended in these special patient populations unless potential benefits outweigh the potential risks [70].

At present, ITRA solution, in a dosage of 200 mg once daily or 100 mg twice daily for 1 week, repeated as necessary, is indicated solely for the treatment of oral and esophageal candidosis in HIV-positive or immunocompromised adults.

'New' Molecules

Echinocandins: Caspofungin and Anidulafungin

Pneumocandins and echinocandins represent an entirely new class of antifungal molecules. Caspofungin (Cancidas, Merck) is currently the only available echinocandin. Micafungin (Fujisawa) will probably be introduced into clinical practice in the near future and a related molecule, anidulafungin is in phase II clinical research. Candins operate at two sites in fungal metabolism, *D*-glucan synthesis of the cell wall and ergosterol synthesis [74, 75], acting somewhat more slowly (5–7 h) than polyenes and azoles. The antifungal spectrum of caspofungin involves all yeasts and molds apart from *Cryptococcus* (cell wall lacks *D*-glucan), and it is also active against *Aspergillus*, but activity is decreased compared to voriconazole, and against *P. carinii* and *P. jirovecii* [74, 75].

Pharmacological Profile

Plasma clearance of caspofungin is 10–12 mg/min, protein clearance 96% and elimination half-life is 9–11 h through liver metabolism. Interaction with cyclosporin may result in elevation in liver enzymes [76, 77], and tacrolimus concentrations should be measured. Caspofungin is currently only available as intravenous infusion; the daily dose is 50–70 mg/kg for adults and 70–100 mg/kg for children. In contrast to AmB, no dose reduction in kidney failure is necessary [78].

Clinical Application

Caspofungin was compared to AmB in HIV-positive patients with candidal esophagitis [79] with 89% efficacy (66.7% in AmB-treated patients); caspofungin 50 mg once daily showed 85% efficacy compared to 82% for FLU 200 mg once daily [80]. In a study of refractory (not responding to AmB or other agents) aspergillosis, a 41% response rate was observed in this prognostically inferior group of patients [80]. In the absence of a randomized controlled study on caspofungin versus lipid formulations of AmB or conventional AmB in documented aspergillosis, caspofungin was considered as second-line therapy or may be used in combination with AmB, or voriconazole, for IA [79, 80]. In two further controlled studies [80, 81] involving more than 200 patients with IA, responses [82] of 49 vs. 19% were observed in non-neutropenic patients in favor of caspofungin [81].

The most recent study that compared caspofungin with AmB in the treatment of invasive candidemia was published [83, 84]. Some 224 patients with candidemia were evaluated, and response was 80.7% (caspofungin) and 64.9% (AmB). This is to date the largest candidemia study with caspofungin. Finally, a study with a small group of patients using caspofungin in combination with AmB has been published [85]. Caspofungin was documented to be effective in candidemia or esophageal candidiasis as monotherapy, and for aspergillosis either as a second-line agent for non-responders or in combination with AmB (conventional or lipid formulation). Further studies are expected to evaluate the role of caspofungin in IA as monotherapy as well as in pediatrics, where no studies are available.

Triazoles: Voriconazole, Posaconazole and Ravuconazole

Voriconazole

Voriconazole (UK-109,496), a novel wide-spectrum triazole antifungal agent, is active in vitro against *Aspergillus* species for which the geometric mean MIC is 0.4 mg/l, which compares favorably with that of AmB [86–88]. The drug is fungicidal in vitro for a majority of isolates and can be given both orally and intravenously, making switch therapy easier [86–94]. Several studies evaluated the clinical efficacy and safety of voriconazole in the treatment of acute IA candidemia and also the empiric therapy of IFI in neutropenic and other immunocompromised patients [93–95].

Denning et al. [96] evaluated the efficacy and safety of voriconazole in acute IA in an open, noncomparative multicenter study. Immunocompromised patients with IA were treated with intravenously administered voriconazole 6 mg/kg twice a day twice and then 3 mg/kg twice daily for 6–27 days, followed by 200 mg administered orally twice daily for up to 24 weeks. Response was assessed by clinical and radiographic changes. A total of 116 patients were assessable; IA was proven in 48 (41%) and probable in 68 patients. Voriconazole was given as primary therapy in 60 (52%). Good responses were seen in 56 patients (48%); 16 (14%) showed complete response and 40 (34%) partial response. A stable response was seen in 24 patients (21%), and 36 (31%) patients failed to respond to therapy. Good responses were seen in 60% of those with pulmonary or tracheobronchial IA (n = 84), 16% with cerebral IA (n = 19), 58% with hematologic disorders (n = 67), and 26% of allogeneic stem cell transplant recipients (n = 23).

Herbrecht et al. [97] enrolled a total of 144 patients with definite or probable aspergillosis in a voriconazole treatment group and 133 patients in an AmB group; patients received at least one dose of treatment. In most of the patients the underlying condition was allogeneic hematopoietic-cell transplantation, acute leukemia, or other hematologic diseases. At the 12-week follow-up, there were successful outcomes in 52.8% of the patients in the voriconazole group (complete responses in 20.8% and partial responses in 31.9%) and in 31.6% of those in the AmB group (complete responses in 16.5% and partial responses in 15.0%; absolute difference, 21.2 percentage points; 95% confidence interval, 10.4–32.9). The survival rate at 12 weeks was 70.8% in the voriconazole group and 57.9% in the AmB group (hazard ratio, 0.59; 95% confidence interval, 0.40–0.88). Voriconazole-treated patients had significantly fewer severe drug-related adverse events, but transient visual disturbances were common with voriconazole (occurring in 44.8% of the patients). In patients with IA, initial therapy with voriconazole led to better responses, improved survival and resulted in fewer severe side effects than the standard approach of initial therapy with AmB.

Walsh et al. [98] published a study on empiric therapy of febrile neutropenia with voriconazole in comparison to liposomal AmB. This large study included 415 and 422 patients randomized to receive empiric therapy (either voriconazole or ambisome) for febrile neutropenia not responding to antibiotics. Significantly fewer breakthrough fungemias ($p < 0.02$) overall and in high-risk patients ($p < 0.003$) were observed in those receiving voriconazole.

Posaconazole and Ravuconazole

Posaconazole and ravuconazole are the newest azoles that are currently in phase II–III studies and which will probably come to clinical use in 2005 [99–108].

Posaconazole is a new triazole antifungal agent synthesized by the Schering-Plough Research Institute. Preclinical studies of this agent conducted in various countries, including Japan, have shown excellent in vitro and in vivo antifungal activities [99–103] also against mucormycosis. Before embarking on clinical trials in Japan, a basic study to confirm the in vitro antifungal activities was performed [100, 101]. Ravuconazole showed very good in vitro activity against NAC species. However, results from clinical studies are not currently complete.

New Strategies in the Management of IFI

Antifungal Combination Therapy

Antifungal combination therapy is based on (i) in vitro studies on synergism of particular antifungals, (ii) animal models, and (iii) clinical experience based on case reports, retrospective observational studies or prospective clinical trials. The majority of applications are based on the treatment of fungal infections not responding superficially to standard antifungal therapy (e.g. cryptococcosis, some NAC species) or caused by multiresistant organisms (*C. krusei*, *C. glabrata*, *Fusarium* spp.) and in settings associated with high mortality (aspergillosis). It should be borne in mind that a truly synergistic effect at the level of fungal cells is not the only prerequisite for a beneficial effect in human chemotherapy [109].

The first antifungal combination to be tested in controlled clinical trials was 5-FC + AmB which was studied in patients with cryptococcal meningitis. Meningeal cryptococcosis was chosen because it is always lethal and needs an aggressive, preferentially fungicidal, therapy.

The combination of 5-FC + FLU to treat cryptococcal meningitis in AIDS patients [110] demonstrated clinical success that was significantly greater than previously reported for AmB or FLU monotherapy. A total of 75% of the patients had negative cultures in the cerebrospinal fluid 10 weeks after initiation of therapy, while only 35–40% of the cultures became negative under monotherapy. The overall clinical success rate was 63% with the combination. However, toxic side effects that were sufficiently severe to lead to withdrawal of 5-FC were observed in 28% of the patients. Apart from cryptococcal meningitis, AmB + 5-FC has been used in the treatment of fungemia caused by resistant *Candida* spp. Combination therapy should be used only for cryptococcal meningitis.

Combination therapy with 5-FC + AmB has clearly established its beneficial value for human chemotherapy and is still used for deep-seated mycoses in severely immunosuppressed patients. The interaction of azoles and polyenes clearly varies by fungus, test model and drug. No general rule concerning the concomitant or sequential use of these drugs exists; each case requires individual study [110–112].

Immunomodulators

Various cytokines have been produced using biotechnological methods. Most of these factors have been thor-

oughly investigated in vitro and in vivo to define their role in defense against fungal infections. Some were found to show a depressive effect increasing the acute course of fungal infection, for example interleukin 4 in candidosis [113]. Most immunomodulators, however, have the capacity to increase host defense and therefore cause an increase in survival time or a decrease in the fungal burden [114, 115].

The most promising immunomodulators for fungal infections appear to be the G-CSF, GM-CSF and M-CSF. The available in vitro and animal data clearly show that addition of various immunomodulators augment the beneficial effect of conventional antifungal agents. The data from preliminary human studies combining cytokines with traditional antifungal therapy generally suggest that augmentation of the immune system complements the effect of antifungal therapy. For example, reversal of immunodeficiency is essential for successful recovery from IA. The mortality rate and duration of granulocytopenia can be decreased by the addition of G-CSF or GM-CSF to conventional antifungal therapy, leading to more rapid and complete recovery [113–115].

Most clinical work has been done with the various colony-stimulating factors (G-CSF, GM-CSF and M-CSF). Several encouraging reports exist describing the use of G-CSF in patients with fungal infections and underlying conditions that cause neutrophilic deficiency, such as aplastic anemia or chronic granulomatous disease [116, 117].

Surgical Treatment of IFI

Several IFI cannot be cured just with antifungals even when combined with immunomodulators [118]. (i) In all cases of fungemia related with a foreign body (e.g. shunt infection or prosthetic cardiac valves), the foreign device must be surgically removed. Surgical intervention is essential for a better outcome, reduced duration of fungemia and reduced morbidity and mortality. Antifungal chemotherapy should accompany the surgical intervention. (ii) In metastatic complications of candidemia (e.g. brain or liver abscess) surgical punctio and drainage is essential. (iii) In IA, surgery is important in the treatment of pulmonary diseases which present bleeding complications, and it reduces significantly mortality as a result of aspergillosis erosion of lung vessels. Radical resection of infected lung tissue is often life-saving surgery, however it requires an experienced surgeon and massive hematologic support because of concomitant pancytopenia. (iv) In rhinocerebral or ophthalmic mucormycosis, radical

resection or vitrectomy (ophthalmic) followed by lipid formulations of AmB or voriconazole is the only possible therapy, reducing mortality from 100 to 50–75%. (v) In aspergillosis sinusitis, surgical drainage is not only used to prevent sinoantral IA, but also to prevent the hematogenous spread during subsequent immunosuppression (e.g. in leukemia, bone marrow transplantation and graft-versus-host disease).

Surgery performed in ii–v is an important therapeutic strategy to treat or prevent IFI, and is followed or accompanied by antifungal chemotherapy. Initial (empiric) therapy of IFI in surgical patients has also been studied [118–122].

Specific Strategies in Pediatrics

Several risk factors and the epidemiology of invasive candidiasis are different in children in comparison to adults. Also, the outcome of *Candida* infection in children is better. Pharmacokinetic properties of new azoles are different in small children and especially in neonates compared to adults. In large multicenter studies examining new antifungal agents, data in children and adults are not separately evaluated; therefore it is difficult to assess the activity of those compounds in children. Data from adults may be extrapolated to older children and adolescents but not to neonates. Some retrospective or open studies on FLU and a lipid formulation of AmB in children are available to assess activity and safety of those drugs in the treatment of systemic candidiasis in pediatrics [123–130].

There are differences in the pharmacokinetics of antifungal drugs in children. This phenomenon can be seen in many antimicrobial agents, where the half-life is shorter and *C max* may be lower than in adults. This was documented for FLU [124] as well as the lipid formulation of AmB [128]. Therefore, dosing intervals of FLU in neonates and small children <5 years of age should be shorter [125–126]. Pharmacokinetics of lipid formulations of AmB, mainly of ABLC, was also studied in neonates, where different dosing intervals were examined. Compared to adults, children older than 3 months of age manifest more rapid clearance of AmB [129]. Premature neonates are characterized by extreme individual variation in the distribution and clearance of AmB, and thus individual serum levels cannot be predicted with certainty [129]. Some neonates continue to accumulate AmB during the course of therapy, an observation suggesting that they require a dose interval of more than 24 h. AmB toxicity is less severe in infants and children than in

adults, probably because of the more rapid clearance of the drug in children [126,127]. However, in children >5–10 years old, the pharmacokinetic parameters of antifungals are similar in children and adults. Concerning toxicity, generally liver and kidney toxicity of AmB is lower in children than in adults. Children usually tolerate higher doses of AmB (1.5 µg/kg/day of AmB or 5–10 µg/kg/day of lipid formulations of AmB [127]). The safety of azoles is higher than, or similar to, adults, even in premature neonates. No significant toxicity of higher doses of FLU was observed in children [123–125].

In vivo and in vitro Correlation

Because of advances in therapeutic interventions as well as the AIDS epidemic, rates of fungal infections have shown manifold increases in recent years [131, 132]. While AmB and 5-FU were for a long time the only available systemic antifungal agents, both of these drugs are difficult to use; the pharmaceutical industry has responded to the demand for new therapies for fungal infections with the introduction of ketoconazole, ITRA, FLU, and the lipid formulations of AmB. The National Committee for Clinical Laboratory Standards has proposed standardized methodology for antifungal susceptibility testing and tentative interpretative breakpoints for the triazole agents. However, the methodology remains to be validated for AmB testing [3, 18, 19].

Unlike in vitro antibacterial susceptibility testing, which has been useful in the selection of optimal antibacterial therapy, antifungal susceptibility testing has been hampered by a number of limitations, including the lack of standardized methodology and in many cases the lack of correlation between in vitro results and in vivo outcomes [5, 131–133].

There have been several studies showing partial or entire correlation between resistance and infection outcome. However, the majority of these studies [4, 6, 19] documented this relationship on HIV-positive individuals with candidiasis in the setting of patients treated with FLU. Other factors associated with inferior outcome (therapy failure or relapse) were prior therapy with FLU, CD4 cell count, and progression of AIDS.

In all those studies, patients with MICs to FLU of 32 or 64 µg/ml and more, and even in the group with MICs of 8–16 µg/ml, were associated with more failures, prolonged therapy and more relapses. Increasing the dose of FLU to 400–600 mg/day showed no effect on outcome, because the relapses occurred late. Some of those studies

assessed the MICs to other antifungal agents such as AmB, 5-FU or ITRA. However, at least two other studies in HIV-negative patients (one cancer and one surgery + intensive care unit patients) were published in 1995–1998 and demonstrated an in vitro-in vivo correlation in proven fungal infection (fungemia) [12].

In the first study, Nguyen et al. [3] examined more than 100 fungemias in cancer patients caused by *Candida* spp. and found two correlations between the in vitro susceptibility and clinical outcome. Cases with minimal bacterial inhibition for AmB of 1 µg/ml and those with MLC ≥ 0.5 µg/ml were both associated with an inferior outcome. This correlation was highly significant ($p < 0.001$) for minimal bacterial inhibition and MLC, but not for MIC.

In the second study, collecting 264 fungemias in intensive care unit and surgery patients including those with cancer (mixed patient population), patients with candidemia caused by organisms with MICs to FLU > 32 µg/ml had higher mortality than individuals infected with susceptible *Candida* spp. [12].

Beyond the last two fungemia studies (with 100 and 264 cases), the largest multicenter fungemia study in the US was only for in vitro-in vivo correlation among more than 200 candidemias in cancer patients [5]. The authors even observed that patients infected with *Candida* spp. with higher MICs to AmB and FLU had better outcomes. However, this association (which may be a surprising one) was statistically insignificant. Therefore, the only correlation drawn from that study was an absence of the relationship of MICs to FLU and AmB and the clinical outcome of candidemia in both small and attributable mortality.

Finally, a large multicenter study testing more than 1,000 bloodstream isolates of *Candida* spp. was conducted in the US by Pfaller and the Mycoses Study Group [13]. Despite a correlation in vitro to in vivo activity, no trends towards an increase of resistance to FLU in *Candida* spp. within the last 5 years was observed. Several other studies observed stable resistance to AmB and FLU despite 50 (AmB) or 15 (FLU) years of clinical use, but correlations of clinical outcome and resistance were not studied [131, 132].

Conclusions

In this review, only some of the current therapeutic promises and dilemmas in the management of IFI have been discussed. Some hope has been observed with respect to outcome. After a decade of ‘standstill’ in the antifungal armamentarium, several agents are in the pipeline of development in the pharmaceutical industry. However, only two appear to influence mortality (voriconazole and lipid formulations of AmB). Fortunately, at least one molecule has shown an impact on outcome, which is the ultimate goal in the development of antifungal agents.

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