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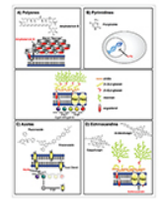

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REVIEW

Antifungal therapeutics for dimorphic fungal pathogens

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ABSTRACT

Dimorphic fungi cause several endemic mycoses which range from subclinical respiratory infections to life-threatening systemic disease. Pathogenic-phase cells of *Histoplasma, Blastomyces, Paracoccidioides* and *Coccidioides* escape elimination by the innate immune response with control ultimately requiring activation of cell-mediated immunity. Clinical management of disease relies primarily on antifungal compounds; however, dimorphic fungal pathogens create a number of challenges for antifungal drug therapy. In addition to the drug toxicity issues known for current antifungals, barriers to efficient drug treatment of dimorphic fungal infections include natural resistance to the echinocandins, residence of fungal cells within immune cells, the requirement for systemic delivery of drugs, prolonged treatment times, potential for latent infections, and lack of optimized standardized methodology for in vitro testing of drug susceptibilities. This review will highlight recent advances, current therapeutic options, and new compounds on the horizon for treating infections by dimorphic fungal pathogens.

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Introduction

Within the Ascomycetes phylum, members of the genera *Histoplasma, Blastomyces, Coccidioides*, and *Paracoccidioides* are dimorphic fungi which are responsible for hundreds of thousands of infections, many thousands of which require clinical intervention. Given similarities in their pathogenesis, the disease manifestations they produce, their clinical management, and particularly the resemblance of their antifungal susceptibility profiles, these 4 genera will be the subject of this review. Two additional dimorphic fungi, *Talaromyces marneffei* and *Sporothrix schenkii*, will not be covered. Neither dimorphic fungi nor human pathogens are monophyletic groups within the Onygenales as closely related non-pathogenic and non-dimorphic species exist for several of these genera. While once designated as a single *Coccidioides* species, *Coccidioides immitis*, advances in sequence analyses and phylogenetics have separated it into 2 species: *Coccidioides immitis* and *Coccidioides posadasii*.[1] Other dimorphic fungi have similarly been separated into different species with *Paracoccidioides braziliensis* now comprised of *Paracoccidioides brasiliensis* and *Paracoccidioides lutzii*[2] and *Blastomyces* dermatitidis as the 2 species *Blastomyces dermatitidis* and *Blastomyces gilchristii*.[3] From a clinical standpoint, management of these diseases does not rely on knowing the precise species. Thus, throughout this review, the dimorphic fungal pathogens will be referred to by their genus name without regard to the specific taxonomic species designation. Both current antifungals and potential new drugs for treating infections by dimorphic fungal pathogens will be discussed.

Dimorphism and endemicity

The hallmark of the dimorphic fungal pathogens is their dual, yet distinct lifestyles. These lifestyles are correlated with separate fungal morphologies with filamentous growth (i.e., hyphae) characterizing the saprobic phase in the environment and yeasts (or spherules in the case of *Coccidioides*) characterizing the human pathogenic phase. This contrasts with polymorphic fungi (e.g. *Candida albicans*) which exhibit both yeast and hyphal morphologies in human tissues. Temperature appears to be the central factor that determines the form/lifestyle of the dimorphic fungi with exposure to mammalian body temperature triggering adoption of the pathogenic mode.[4-7] Yeasts of the dimorphic fungi (or *Coccidoides* spherules) are not efficiently eliminated by immune cells. Preventing the transition into yeasts through pharmacologic[8,9] or genetic interventions[10-13] renders dimorphic fungal pathogens avirulent indicating the necessity of the dimorphic transition for pathogenesis. Unlike many parasites which must cycle between human and non-human

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environments to complete their lifecycle, the dimorphic fungi have no such requirement for infection of mammalian hosts. Rather, infection of mammals by the dimorphic fungi is accidental, yet when these fungi transition to the yeast/spherule phase, they express efficient mechanisms that enable their survival and proliferation within this secondary environment.

The dimorphic fungal pathogens are endemic to particular geographic regions rather than being found ubiquitously in the environment. These areas have been largely defined by clinical case prevalence,[14] isolation from soils,[15] or skin-reactivity tests to specific antigens of the dimorphic fungi.[16-18] Ecological aspects of soils that favor growth of the hyphal forms and/or animal or bird patterns for dispersal are thought to underlie dimorphic fungal pathogen endemicity. *Histoplasma* is endemic to the Midwest and Eastern parts of the United States (chiefly around the Ohio and Mississippi River valleys),[16] and is also found in Latin America (particularly Brazil, Venezuela, Colombia, and Argentina;[19]), parts of Africa,[20] and some cases have been reported in China[21,22] and India.[19] *Blastomyces* overlaps with many of the *Histoplasma*-endemic regions in the United States (Midwest, Southeast, and South-central) and also includes Canadian provinces around the Great Lakes[23,24] and parts of Africa.[25] In contrast to the moist environments with decaying organic material supporting *Histoplasma* and *Blastomyces, Coccidioides* is found in the more arid environments of the Southwest of the United States (Southern California, Arizona, New Mexico, Texas;[26]), Central America, and parts of South America (Northern Brazil, Venezuela, Argentina, and Paraguay[27]). *Paracoccidioides* species are endemic to South America, particularly areas of Brazil, Colombia, Venezuela, Argentina, and Uruguay.[28]

The annual incidence of infections by dimorphic fungi is likely inaccurate as under-diagnosis and under-reporting of infections is common. Furthermore, most infections are self limiting without requiring clinical intervention. Nevertheless, estimates of over a half million infections by *Histoplasma* and *Coccidioides* occur each year.[29,30] *Paracoccidioides* and *Blastomyces* infection estimates are more difficult due to regional differences, however reports suggest the incidence ranges from 0.05 to 3 per 100,000 individuals in endemic regions.[23,31-33] Together these 4 dimorphic fungal pathogens infect hundreds of thousands each year, a number which does not include veterinary infections. The number of clinical cases typically range from 5–30% of infections, although in some outbreaks over 50% of infections can result in clinical disease.[34-36] One survey of hospital records in the United States tallied over 6000 hospitalizations in one year: 3360 cases of histoplasmosis, 771 cases of blastomycosis, and 2194 cases of coccidiodomycosis.[14] Less than half of these were in immunocompromised individuals. A case series analysis of Latin America countries showed over 750 annual reported cases of paracoccidioidomycosis.[28]

Dimorphic fungal pathogen disease and treatment

Inhalation of conidia initiates infection by the dimorphic fungi[28,37-39] The conidia (or arthroconidia in the case of *Coccidioides*) are produced solely by the environmental mycelia. The small size of these propagules facilitates aerosolization upon environmental disturbance and deposition into alveoli once inhaled. Thus, occupational and recreational activities that contribute to soil and environmental disruption are a primary risk factor for infection by dimorphic fungal pathogens.[39-42] Most infection outbreaks can be traced back to specific events that led to conidia release from soils and their subsequent inhalation.[34,35,43-47] Therefore, practices to avoid or reduce the potential for aerosolization and inhalation of conidia are prescribed for high risk areas[34,41,48,49]

Within the lungs, conidia transition into pathogenic-phase cells which survive innate immune defenses. For *Histoplasma, Blastomyces*, and *Paracoccidioides*, the conidia germinate into yeasts. The yeasts can be internalized by host phagocytes (e.g.,, alveolar macrophages) to varying degrees with *Histoplasma* yeasts residing almost exclusively within these host cells. This intracellular residence creates an additional barrier to antifungal drug penetration which must be considered in antifungal development. For *Coccidioides*, the inhaled arthroconidia become spherules that are exclusively extracellular. The spherules enlarge into endosporulating structures within the lung tissue and release endospores for propagation of the infection. Whether phagocytosed or not, the dimorphic fungal pathogens are not controlled by the innate axis of the immune system unlike the opportunistic fungal pathogens. Thus, *Histoplasma, Blastomyces, Coccidioides* species, and *Paracoccidioides* infections are not restricted to immunocompromised hosts, but also cause disease in immunocompetent individuals. Control of the infection requires activation of $CD4^+$ cells and consequently individuals lacking aspects of cellular immunity (e.g., HIV, immunosuppression due to tissue or organ transplantation, TNFα blockade, etc.) typically progress to severe and disseminated disease. Elimination of symptoms has been assumed to indicate clearance of the infection, but evidence is now suggesting that at least in some individuals, the infection can enter a latent state, which can re-emerge later when the balance between pathogen and host immunity is altered (e.g., immunosuppression of the host).[50-56]

Since inhalation is the route of exposure, mycoses caused by dimorphic fungal pathogens are initially pulmonary diseases. In immunocompetent individuals, mild disease is mostly subclinical, often going undiagnosed. Infection causes varying degrees of pneumonia and influenza-like symptoms. For the majority of individuals, symptoms typically resolve without requiring intervention. Roughly 5% of *Histoplasma* infections are estimated to require clinical management[57] and up to 30% for *Coccidioides* infections.[58] For individuals inhaling a larger inoculum, or those that have some deficiency in cellular immune response, disease is more severe and the infection typically disseminates to extrapulmonary sites via the hemolymphatic system. Extrapulmonary disease presentation varies, but can include oral and pharyngeal mucosa (*Paracoccidioides*), cutaneous lesions (*Histoplasma, Blastomyces, Paracoccidioides*, and *Coccidioides*), and bone (*Blastomyces*).[31,38-40,59] The systemic nature of infections by dimorphic fungi thus precludes any topical antifungal options for management, and the required systemic drug administration significantly elevates the potential for problems with host toxicities.

Current antifungal options

In vitro activities of current antifungal drugs used clinically have been established for the dimorphic fungi. These include drugs of the polyene, azole, and echinocandin classes. In general, reference methods for antimicrobial susceptibility tests follow those established by the Clinical and Laboratory Standards Institute (CLSI). Notably missing from these reference methods are procedures specifically designated for dimorphic fungi leading to confusion as to whether macro- and microdilution test methods for yeasts (M27-A3)[60] or for filamentous fungi (M38-A2)[61] should be followed. This is not an insignificant question as the yeast and hyphal forms of the dimorphic fungi can have dramatically different susceptibilities.[62-69] For example, early studies with echinocandins showed antifungal effects on the hyphal form of *Histoplasma*.[67] However, subsequent tests against the yeast form indicated that caspofungin was anywhere from 20- to 1000-fold less effective.[64,68,69] It is now well recognized that the echinocandins have poor antifungal activity on pathogenic phase cells of the dimorphic fungi. This situation underscores the need to use the form most relevant to human infection when establishing the antifungal susceptibility profile of dimorphic fungal pathogens. Unfortunately, some antifungal studies continue to ignore the pathogenic forms and instead limit studies to mycelial-phase cells.[70-72] Further complicating antifungal susceptibility profiling are the lack of standardized methods addressing complications for dimorphic fungi. While the CLSI methods for yeasts (M27-A3)[60] work well for *Candida* and *Cryptococcus* yeasts, they are inadequate for testing yeast-phase cells of the dimorphic fungal pathogens, which have longer generation times and require higher inocula for efficient and consistent growth in broth culture. A more appropriate microdilution method has recently been established for antifungal testing of *Histoplasma* yeasts,[69] and this methodology produces more accurate data for *Histoplasma* susceptibility.[73]

Despite these caveats, a general profile of the antimicrobial susceptibilities of dimorphic fungi has been developed for amphotericin B and azole-class drugs. Each of the dimorphic fungal pathogens exhibit a similar susceptibility profile for current antifungals with both amphotericin B and azole drugs generally showing potent activity (low minimum inhibitory concentrations; MIC) with some variability in MICs reported for fluconazole (Table 1). Unfortunately,

Table 1. In vitro antifungal MICs for dimorphic fungal pathogens.

		MIC range (μg/mL)				
Drug class	Antifungal	*Histoplasma*	*Blastomyces*	*Paracoccidioides*	*Coccidioides*	References
Polyenes	Amphotericin B	Y: <0.03–2.0 M: 0.26–2.5	Y:<0.03–2.0 M:	Y: 0.06–2.0 M:	Y: 0.25–2.0 M: 0.03–0.50	68,69,109-119
Imidazoles	Ketoconazole	Y: M: 0.17	Y: <0.01–0.25 M: 0.1–0.4	Y: <0.01–0.03 M:	Y: M: 0.03–0.16	72,110,114-116,120
Triazoles	Fluconazole	Y: 0.25–8.0 M: 2.0–32	Y: 0.06–32 M: 0.06–32	Y: 0.13–0.50 M:	Y: M: 2.0–64	68,69,109,110,112-114,116,117,119-123
	Itraconazole	Y: <0.01–0.5 M: 0.03–1.0	Y:<0.01–0.13 M: 0.03–4	Y: <0.01–0.06 M:	Y: <0.03–0.50 M: 0.03–1.0	68,109-115,120-122
	Voriconazole	Y: 0.03–0.50 M: <0.01–2.0	Y:<0.03–0.25 M: 0.06–2.0	Y: M:	Y: <0.03–2.0 M: 0.03–1.0	109-111,113,121,123
	Posaconazole	Y: <0.01–0.50 M: 0.02–2.0	Y: <0.02–0.06 M: <0.02–2.0	Y: M:	Y: M: 0.06–1.0	67,109,112,121,123
Echinocandins	Micafungin	Y: >64 M: 0.03–0.06	Y: 32–64 M: <0.01–0.03	Y: >64 M: 4–16	Y: M: 0.02	68
	Caspofungin	Y: 8–32[a] M: 0.02–4.0	Y: M: 0.5–8.0	Y: M:	Y: M: 8–64	67,69,113,117,118

Note. [a]2 studies reported low caspofungin MICs for yeast which disagree with the majority of studies based on clinical isolates in India (0.03–1.0 μg/mL)[109] and for a single laboratory *Histoplasma* strain (MIC <0.125 μg/mL).[122]

the echinocandins, which have dramatically decreased host toxicity, have poor antifungal activity against the pathogenic-phase cells of dimorphic fungal pathogen yeast cells. The reasons underlying this natural resistance of yeasts to the β-glucan synthase inhibitors is currently unknown.

The in vitro antifungal susceptibilities for amphotericin B and azole-class drugs have been validated in murine models of dimorphic fungal disease and/or in clinical trials. As a consequence, the Infectious Disease Society of America has released treatment guidelines for infections with *Histoplasma*,[74] *Blastomyces*,[75] and *Coccidioides*.[76] Despite its potential for host toxicity, amphotericin B is recommended for severe disseminated disease, with one of the liposomal formulations preferred. For less severe situations, for follow up after amphotericin B, or for possible prophylaxis of highly susceptible individuals (e.g. AIDS), IDSA guidelines recommend treatment with an oral azole (i.e., itraconazole) with monitoring of serum concentrations to ensure sufficient absorption and bioavailability. To ensure sufficient clearance of the dimorphic fungal infection, treatments typically involve protracted regimens. For mild disease, treatment durations can range from several months to a year depending on the specific dimorphic fungus. Treatment of disseminated disease and disease in immunocompromised hosts can be a year or longer. Beyond the prolonged treatment times, the potential for latency, rather than clearance of the infection, further complicates antifungal management of dimorphic fungal infections.

New and alternative antifungal developments

With the known host toxicity risks of current antifungals,[77-79] and the endogenous resistance of dimorphic fungi to the lower toxicity echinocandins, new and alternative antifungal drug options have been explored. The eukaryotic nature shared by both the dimorphic fungal pathogens and the host is recognized as a significant obstacle to antifungal drug development. Consequently, many molecules with promising antifungal activity fail due to lack of adequate selectivity.

One approach to improve the selectivity of existing antifungal molecules, is to test modifications to the structure that decrease drug affinity to host targets. The oral availability of azole-class antifungals is a great benefit to management of dimorphic fungal infections. However, the heme iron-binding action of the imidazoles and triazoles that inhibits fungal cytochrome CYP51 (Erg11) also targets host P450 cytochromes leading to hepatotoxicity and problematic drug-drug interactions. Exploration of other metal binding azole groups in combination with structure-guided improvement of affinity for fungal CYP51 outside of the active site led to the design of compound VT-1161.[80] As predicted from structure affinity calculations, the molecule has low affinity for mammalian CYP3A4 but is highly potent against *C. albicans*. Subsequent testing against *Coccidioides* arthroconidia in vitro showed VT-1161 has an MIC around 1.5 μg/mL.[81] Oral administration of VT-1161 in a murine model of coccidioidomycosis demonstrated VT-1161 reduced spherule burden in the lungs by 100- to 1000-fold, leading to improved mouse survival.[81] While VT-1161 had similar effectiveness as fluconazole in vivo, it is hoped the better selectivity of the molecules will decrease potential host toxicity.

Following the goal of reduced host toxicity, some antibacterial compounds have been investigated due to their established safety profile to the host. Drugs targeting the folate pathway have been effective against microbes from bacteria to parasites,[82] are orally available, and have a moderately safe host toxicity profile. These drugs have already been a means of prophylaxis against *Pneumocystis* fungal infections in HIV+ individuals. Sulfonamides in combination with dihydrofolate reductase inhibitors (e.g., cotrimoxazole) have activity against *Histoplasma* yeasts (8–14 μg/mL and 2–3 μg/mL, respectively)[65] and *Coccidioides* (MICs of 1000 ug/mL and 200 ug/mL, respectively),[66] although only the mycelial phase has been tested for *Coccidioides*. The sulfonamides/antifolate combination has been one of the traditional treatments for *Paracoccidioides*[59,83-85] but they have not been advanced clinically for the other dimorphic fungal pathogens.

Using a similar rationale, ciprofloxacin, a fluoroquinolone antibacterial with very low host toxicity, has been tested for antifungal potential. The fluoroquinolones inhibit bacterial type II topoisomerases (e.g., DNA gyrase) and were suggested to potentially also inhibit fungal topoisomerase. While not effective against mycelial forms of *Histoplasma* and *Coccidioides*, tests against *Histoplasma* yeast showed ciprofloxacin has an in vitro MIC of 62.5–250 μg/mL.[86] While not considered a low MIC, the lack of significant host toxicity for ciprofloxacin creates a good selectivity index and raises its potential, at least as an adjunct therapy for fungal infections. Addition of ciprofloxacin to amphotericin B or itraconazole indicated some additive effects could be gained through drug combinations.

Anti-tuberculosis drugs show some, albeit low potency, antifungal activity for dimorphic fungal pathogens. When tested in vitro against *Coccidioides* mycelia, rifampicin, isoniazid, and ethambutol had very high MICs (8500 μg/mL, 500 μg/mL, and 2500 μg/mL, respectively[72]). Effectiveness against spherules was not determined but tests against *Histoplasma* yeasts similarly had high MICs[71] arguing these antibacterial compounds lack any significant antifungal utility. However, using isoniazid as the core, chemical structure modification improved its antifungal potential; 3 of 9 isoniazid derivatives had significantly lower MICs against

Histoplasma yeasts with one having inhibitory activity at concentrations as low as 7.8 μg/mL.[62] Extending the in vitro tests of the isoniazid-hydrazone derivative to *Coccidioides* demonstrated it had an MIC of 50 μg/mL against mycelia of this dimorphic fungal pathogen.[87] Although *Coccidioides* spherules were not tested, there is a reasonable possibility that the MIC will be lower since *Histoplasma* yeasts were 8–16X more sensitive to the isoniazid-hydrazone than were mycelia.[62]

Antiretroviral protease inhibitors have inhibitory activity against *Histoplasma*. Saquinavir is active against both filamentous and yeast forms with an MIC around 0.4 μg/mL.[63] Another protease inhibitor, ritonavir, has an MIC of 1.0 μg/mL against Histoplasma mycelia but is 7-fold more potent against yeast cells. As HIV+ individuals comprise a significant portion of clinical *Histoplasma* cases, this raises the possibility that management of HIV through protease inhibitor cocktails could simultaneously directly combat infection by dimorphic fungal pathogens.

Surprisingly, farnesol can inhibit growth and morphological transitions of *Paracoccidioides* and *Coccidioides*. Farnesol is an alcohol present in some essential oils but is also produced by *C. albicans* as a quorum sensing molecule. When tested on *Paracoccidioides* yeasts, farnesol had an MIC of 30 μM.[88] Farnesol also inhibited the transition from yeast cells to hyphae, although this morphological defect has questionable relevance to the situation in mammalian hosts. Against *Coccidioides* mycelia, farnesol was nearly 100-fold more potent (MIC approximately 0.3 μM;[70]). Farnesol was also reported to inhibit *Histoplasma* yeast growth with an average MIC of 0.02 μM.[89] As a caution, tests with *Histoplasma* yeasts followed the CLSI methodology (M27-A3) and this has been shown to be inferior for reliable testing of *Histoplasma* yeast susceptibility.[69]

Repurposing of anti-cancer drugs has also been fruitful in the search for new antifungal prospects. AR-12 is a celecoxib-derivative that has been shown to have antimicrobial properties against microbes ranging from bacteria to parasites to fungi and even viruses.[90-94] Early mechanistic studies in cancer cells suggested AR-12 inhibited cellular phosphoinositide-dependent kinase-1 (Pdk1), however this does not appear to be the case in fungi. Recently, it was shown that AR-12 inhibits acetyl-CoA synthetase, an essential enzyme in fungi.[95] AR-12 has antifungal activity against a broad collection of fungi, including the dimorphic fungal pathogens at concentrations around 4–8 μg/mL.[73] In vitro, AR-12 is fungicidal against *Histoplasma* yeasts (Rappleye CA, personal communication). These antifungal characteristics combined with AR-12s safety as established in Phase I trials make AR-12 an attractive antifungal candidate for further development.

Both phenotypic and target-based screening approaches have been used to identify novel compounds that inhibit dimorphic fungal pathogens. Genome analyses for potential antifungal targets focused on those genes that were (i) demonstrated to be essential for fungal growth and (ii) conserved among diverse fungi (Basidiomycetes to Ascomycetes;[96]). From this thioredoxin reductase (Trr) was selected as a candidate target for computational screening of a library of 3000 compounds. Three compounds were identified and tests against purified *Paracoccidioides* Trr protein confirmed the compound inhibited Trr enzymatic activity.[97] Phenotype tests demonstrated that 2 compounds had significant biological activity against *Paracoccidioides* yeasts with MICs of 8–16 μg/mL (compounds F1806-0122 and F3307-0100). This study validates target based screening as an approach to identification of potential antifungal compounds.

Phenotypic screening of small molecule libraries has identified a group of aminothiazoles with antifungal activity toward *Histoplasma*. To enable rapid quantitative screening of yeast growth, yeasts were engineered to express a red-fluorescent protein, which could be used as a surrogate indicator of the number of yeast cells.[98] Furthermore, fluorescence-based growth analysis facilitated efficient monitoring of *Histoplasma* within macrophages, and thus the determination of the effectiveness of compounds on inhibition of Histoplasma within its host cell environment. The top hit aminothiazole (41F5) was effective against yeasts in culture (MIC 2–4 μM) and effectively inhibited proliferation of yeasts resident within macrophages.[98] Importantly, the aminothiazole compound had low toxicity to cultured mammalian cells, including macrophages. Unfortunately, the aminothiazole was not active against *Blastomyces* yeasts despite the very close phylogenetic relationship between *Histoplasma* and *Blastomyces*. The molecular target of the aminothiazole remains to be determined, and this may facilitate structure optimization to broaden its activity for other dimorphic fungal pathogens.

The most advanced new antifungal option for clinical development to manage dimorphic fungal pathogens is nikkomycin Z. The polyoxins (from which the nikkomycins are derived) are peptide modified-nucleoside analogs which were originally identified by screening *Streptomyces* products for antifungal and insecticidal activities.[99] These compounds were later shown to be inhibitors of chitin synthesis.[100] Since chitin synthase is absent from mammalian cells, these compounds have very high selectivity for fungi. Following polyoxin studies on *C. albicans*, the nikkomycins were demonstrated to have good potency in vitro against dimorphic fungal pathogens,[101-103] with the most potency against *Coccidioides* cells (MIC of 0.125 μg/mL for spherules).[103] Examination of treated *Coccidioides* spherules showed lack of endosporulation and even lysis

of the spherules consistent with impairments in cell wall structure.[103] The nikkomycins are orally available, simplifying extension of studies to animal models of mycoses caused by dimorphic fungal pathogens. Administration of nikkomycin Z in murine models of lethal histoplasmosis resulted in decreased organ fungal burdens (3- to 28-fold) and a concomitant enhancement of mouse survival at doses from 20 to 100 mg/kg per day.[102-104] Significant protection from lethal infection was realized, however, only at moderate inocula suggesting nikkomycin antifungal activity may not be sufficiently rapid to curb severe infections. Similar results were found for treatment of blastomycosis with reduction in fungal burdens ranging from 10-fold (at a dose of 50 mg/kg)[103] to apparent lung tissue sterilization (over 3500-fold reduction at higher doses of 400–1000 mg/kg).[105] The ability to safely administer such doses is a testament to the high selectivity gained by targeting the fungi-specific molecule chitin synthase. Nikkomycin Z is also effective in treating pulmonary coccidioidomycosis with over 10,000-fold reduction in lung fungal burdens and complete protection from lethal infection of mice at a dose of 20 mg/kg.[103] Recently, a small clinical trial in dogs with *Coccidioides* infection was completed. Dogs with mild to moderate disease had significant improvement but those with severe disease had little or poor responses to treatment, again suggesting nikkomycin may not act rapidly enough to quickly curtail disease progression in high fungal burden situations. Nonetheless, nikkomycin Z shows good promise as a highly selective (low host toxicity) antifungal for development against dimorphic fungal pathogen infections.

Conclusions

Current clinical management of infections by dimorphic fungal pathogens is limited to azole-class antifungal drugs and amphotericin B. While orally available, the azoles are not without host toxicity issues and the treatment course is lengthy for infections by dimorphic fungi. Development of resistance to azoles is not widespread, although treatment failures due to azole resistance have occurred.[106-108] Unfortunately, the better tolerated echinocandin antifungals lack efficacy against the pathogenic-phase of the dimorphic fungal pathogens raising the need for alternative or second-line treatment options.

While a number of strides have been made in repurposing existing drugs and development of new inhibitors of fungal growth, careful attention must be paid to challenges posed by dimorphic fungi. As the yeasts/spherules of the dimorphic fungi are the state present within the mammalian host, antimicrobial susceptibilities need to be performed with these pathogenic-phase cells, not the mycelia which has led to erroneous conclusions. Testing of the pathogenic-phase in vitro should follow recently optimized procedures as the CLSI methodology for yeasts is inadequate for the dimorphic fungi. Since yeast cells of the dimorphic fungi reside within host phagocytes, it is also advisable for in vitro tests to be followed with tests on drug effectiveness on intracellular yeasts, at least during initial drug development stages.

The overall selectivity of antifungal drug candidates is critical for progression of drugs through the development pipeline. Structure-guided rational design is one approach that has improved the selectivity of an azole structure (VT-1161). Many of the repurposed drugs have relatively high MICs (greater than 100 ug/mL) questioning their therapeutic utility, however if their selectivity is sufficiently high, formulations may be developed to facilitate sufficiently high serum and tissue levels. Lower MICs have been found for drugs targeting the folate pathway, an isoniazid-hydrazone derivative, antiretroviral protease inhibitors, and the anti-cancer drug AR-12, all of which are expected to be reasonably well-tolerated by the mammalian host. Novel drugs with good in vitro MICs and good selectivity include thioredoxin-reductase inhibitors, an aminothiazole compound, and nikkomycin Z. Since nikkomycin Z targets an enzyme absent from the host, nikkomycin has an excellent basis for high selectivity for fungi. In addition, nikkomycin Z has maintained antifungal effectiveness against multiple dimorphic fungal pathogens in animal models of disease. While the current antifungal armament is limited, there are exciting prospects on the horizon for treating dimorphic fungal infections.

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