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General review

Combination therapy in Mucormycosis: Current evidence from the world literature, a mini review

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ABSTRACT

The emergence of Mucorales infections is an urgent global public health threat rapidly disseminating during the current COVID-19 pandemic. Invasive mucormycosis carries significant morbidity and mortality; this is further compounded by the lack of newer effective antifungals on the horizon. Liposomal Amphotericin (L-AMB) is currently considered the cornerstone of antifungals therapy against mucormycosis; However, two decades later (since the introduction of L-AMB), the outcome remains dismal. Furthermore, adverse events related to therapeutic doses of L-AMB are also a hindrance. There is an imperative need for an alternative therapeutic approach to reduce the high mortality. One such approach is to combine the amphotericin with other agents (e.g., caspofungin, posaconazole, isavuconazole, and iron chelators) that can work synergistically or help in reducing the therapeutic doses of L-AMB. This review aims to highlight the various treatment approaches by gathering the clinical evidence from the literature and considering all potential pharmacological combinations that can provide the direction for future studies.

Introduction

In the wake of the COVID-19 pandemic, the burden of invasive mold infections resurfaces. Widespread use of immunosuppressants, poorly controlled diabetes, immune dysregulation by COVID-19, and antibiotic use predisposing to fungal colonization are the various factors attributing to the rise in fungal infections. Mucorales are the group of filamentous molds characterized by aseptate or minimally septate hyphae and angioinvasive disease. This angiotropism results in vascular thrombosis, ischemia and necrosis, causing increased morbidity and mortality [1]. Invasive mucormycosis usually manifests in rhino-orbital-cerebral (ROC) and pulmonary disease. Despite the prompt diagnosis and early incorporation of surgery with antifungals, the mortality remains high. For pulmonary disease, the mortality rate reaches up to 57% [2], whereas in ROC disease, the mortality ranges from 25%–62% [3], which is significantly higher compared to other invasive mold (aspergillosis) infections (45%), [4]. Uncontrolled blood glucose is the major risk factor for ROC disease, whereas hematological malignancy, corticosteroid use and solid organ transplant are the major risk factors for pulmonary mucormycosis [1].

Early aggressive surgery and Amphotericin B (AMB) remain a pivotal components of mucormycosis management. The emergence of AMB resistance in Mucorales is concerning that necessitates the need for novel antifungals and combinations [5]. The introduction of newer azoles like posaconazole and isavuconazole raised some hope. However, the results are disappointing so far. Furthermore, triazoles have been used widely in agriculture, especially in European countries, contributing to a slow rise in drug resistance [6]. Combination therapy in mucormycosis is still unknown territory, with very few studies conducted so far [7-10]. The rarity of the disease, sparse funds and ethical issues regarding the use of newer combinations are the various factors contributing to the paucity of data. Amidst this COVID-19 pandemic with a surge in invasive mold infections, new possibilities should be explored to use combination therapies.

Methods

A literature search was performed on various electronic databases (PubMed, Google Scholar and Scopus) until March 2022. The following keywords were used in the literature search 'mucormycosis', 'Mucorales infections', 'combination antifungals', 'amphotericin', and 'azole' in various combinations. Only articles in the English language were included, and abstracts, posters and editorials were excluded.

Current state of pharmacological management in mucormycosis

In the last two decades, liposomal amphotericin B (L-AMB) has been the drug of choice for invasive mucormycosis. It acts by binding to ergosterol in the fungal cell membrane, causing pore formation, ion leakage and cell death. The doses of AMB range from 5 to 10 mg/

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kg according to The European Confederation of Medical Mycology (ECMM) and Mycoses Study Group Education & Research Consortium (MSG ERC) guidelines. The higher doses are usually recommended in CNS mucormycosis [11]. However, doses >10 mg/kg/day typically do not achieve higher serum concentration with an added risk of nephrotoxicity [12].

The advent of newer triazoles has added a new chapter to the management of mucormycosis. Triazoles inhibit the lanosterol 14α-demethylase resulting in ergosterol synthesis. Posaconazole is a broad-spectrum azole that demonstrated in vitro and in vivo efficacy comparable to AMB [11]. It is currently recommended as a step-down or salvage therapy (300 mg BID on day 1, followed by 300 mg OD) [11]. There is some favourable clinical evidence as an alternative to AMB [13]. However, no head-to-head trial was ever conducted to compare posaconazole with AMB. One retrospective study described a better survival rate with posaconazole compared to L-AMB (92.3% vs 73.4%) in renal transplant recipients (the majority of patients had rhinocerebral diseases and diabetes) [14]. Marty et al. described the promising efficacy of isavuconazole against Mucorales (in vitro/murine model) [15]. It was a single-label, open-arm case-control study (VITAL study) that compared the efficacy of isavuconazole with AMB in matched controls with pulmonary mucormycosis (majority of patients had hematological malignancy). Both groups showed almost a similar 42-day all-cause mortality (33% vs 39%) [15]. However, we must be cautious before making conclusive inferences due to the non-randomized design and small study size [15].

Why we need to explore the novel combination in mucormycosis

The introduction of newer antifungals has changed the landscape of invasive fungal infection over the last decade. However, the early hope was met with disappointment in cases with mucormycosis. L-AMB is the first line antifungal recommended in guidelines for invasive mucormycosis [11]. Combination therapy should be utilized in mucormycosis due to a) High mortality rate, b) the toxicity and dosing difficulty of active antifungal agents (drug-drug interactions, nephrotoxicity, reaching site of infection), and c) the decrease in antifungal susceptibility and possible resistance.

Patients with delayed diagnosis or administration of amphotericin B showed nearly two-fold mortality, especially in pulmonary disease [16,17]. In addition, the use of prophylactic voriconazole has led to selection pressure and the rise of Mucorales infection [18]. This selection pressure increases the risk of mucormycosis and contributes to poor outcomes [18]. According to a multicenter study, a 73% mortality rate was seen in voriconazole-associated mucormycosis [19]. Whether this is a causal association or is there any direct link between voriconazole use and Mucorales hypervirulence is debatable. Based on animal models, voriconazole exposure can cause epigenetic modification (conversion of Mucorales into hypervirulent phenotype), resulting in the upregulation of the efflux pump and subsequent release of virulence factors and changes in sterol composition; that augment the virulence of Mucorales [18]. Furthermore, these cases warrant a high dose of AMB, which is not feasible sometimes due to nephrotoxicity. There is also a concern of cross-resistance to other triazoles, which diminish the efficacy of these drugs [20]. Some Mucorales, especially *Rhizopus arrhizus*, show less susceptibility toward host defense and are challenging to treat. The cell wall of *R. arrhizus* contains more chitin than other fungi, which stimulate cytokines (TNF alpha and IL-6) by mimicking mononuclear cells [21]. Moreover, the R. arrhizus genome is highly repetitive and divergent due to whole-genome duplication (WGD). The gene involved in ergosterol synthesis is the major target of triazoles (e.g., ERG 11 in lanosterol 14α-demethylase synthesis). There are multiple copies of these genes in *R. arrhizus*, which results in variable response and increased virulence [22].

Antimicrobial susceptibility testing and MIC (minimum inhibitory concentration) determination are vital for managing invasive mucormycosis. The recommended reference technique for antifungals susceptibility is the broth microdilution method by the Clinical Laboratory Standards Institute (CLSI) or the (European Committee on Antimicrobial Susceptibility Testing) EUCAST [23]. Gradient concentration strip test (Etest) is now getting recognition as a quick and easy method to determine the antifungals susceptibility [23]. However, agreement of MIC between Reference techniques and Etest is required before the validation of Etest methods. Previous reports had some reservations about the Etest method due to a low level of agreement between reference methods and Etest [24,25]. Of note, a recent study by Vidal et al. described a good correlation between these methods [23]. Though not optimal, Etest can be utilized for antifungals susceptibility testing in Mucorales.

The MIC of triazoles for Mucorales species is gradually rising compared to non-Mucorales attributed to the drug resistance and suboptimal response [26]. The SENTRY antifungal surveillance program published MIC of newer azoles across the various molds. The MIC of posaconazole and isavuconazole was several times high in *R. arrhizus* (2–4 and >8 ug/ml) compared to other molds (e.g., for aspergillus 0.5 and 2 ug/ml). This has led to discordance between optimal fungicidal activity and safe therapeutic drug levels. In addition, it may cause potential therapy failure and necessitates combining the antifungals to overcome resistance [27]. A similar role of antifungal susceptibility for AMB is described by Lamoth et al., and discuss it as an essential determinant of prognosis [28]. They reported better six-week survival in cases with amphotericin MIC <0.5 ug/ml for Mucorales infections. Isolates with MIC >4 ug/ml showed only a 20% response at the end of six weeks [28]. Another murine study by Rodriguez et al. reported the better efficacy of posaconazole in animals infected with *R. arrhizus* that had lower MICs [29]. *Cunninghamella bertholletiae* is a rare Mucorales mainly causing infections in hematological malignancies, and many isolates showed resistance to L-AMB (MIC >8), [29]. This is a primary reason for poor prognosis in patients with *C. bertholletiae* isolates. The MIC data of different antifungals used for the common Mucorales isolates is summarised in table 1 [26,30-32]. Various pathogenic mechanisms and virulence factors that make Mucorales treatment is a unique challenge and responsible for suboptimal antifungal response are depicted in Fig. 1.

Combination therapies in mucormycosis: where are we now ?

Over the last decade, different guidelines advocated only AMB and two of the newer triazole (posaconazole and isavuconazole) for the pharmacological management of invasive mucormycosis. It is imperative to investigate the role of various combinations that can effectively reduce mortality. Combination therapy can target the two different fungal sites (e.g. cell wall and cell membrane), acts in synergism/decrease antagonism and allows to maintain the lower therapeutic drug levels to avoid adverse events. There are some encouraging results in murine and in vitro models. Unfortunately, preclinical results did not reflect in clinical trials so far.

Amphotericin-B with Echinocandins

As earlier discussed, AMB is the most efficacious drug and should be the fulcrum of any potential combination therapy. Echinocandins target beta-(1,3)-D-glucan synthase, whereas the Mucorales cell wall predominantly contains beta-(1,6)-D-glucan synthase, which can explain the ineffectiveness of these drugs [33]. Caspofungin has been shown to inhibit the fungal FKS gene expression (major determinant for echinocandin resistance) and fungal burden [34]. However, no conclusive evidence is shown to support its isolated use in Mucorales treatment. The first murine model that studied the combination therapy was proposed by Spellberg et al. in 2005; they reported increased

Table 1
Summary of MIC data from the literature for common Mucorales isolates .

MIC (mg/L) Mucorale isolates	Antifungals	Borman et al. [26]	Jing et al. [31]	Vitale et al. [32]	Guinea et al. [30]
Mucor spp.	Amphotericin B	0.25	1	0.5	1
	Voriconazole	≥16	>8	>8	>8
	Posaconazole	1	NA	>1	>1
	Isavuconazole.	≥16	8	NA	NA
Rhizopus spp.	Amphotericin B	0.25	1	0.5	1
	Voriconazole	8	8	>8	>8
	Posaconazole	0.5	NA	0.25	0.25
	Isavuconazole.	2	1	NA	NA
Rhizomucor spp.	Amphotericin B	0.25	0.75	0.5	0.5
	Voriconazole	>16	>8	2	>8
	Posaconazole	0.5	NA	0.25	0.5
	Isavuconazole.	NA	1	NA	NA
Lichtheimia corymbifera	Amphotericin B	0.25	0.75	0.25.	1
	Voriconazole	>16	>8	8	>8
	Posaconazole	0.25	NA	0.25	0.25
	Isavuconazole.	4	1	NA	NA
Cunninghamella spp.	Amphotericin B			2	2
	Voriconazole			>8	>8
	Posaconazole			1	0.5
	Isavuconazole.			NA	NA

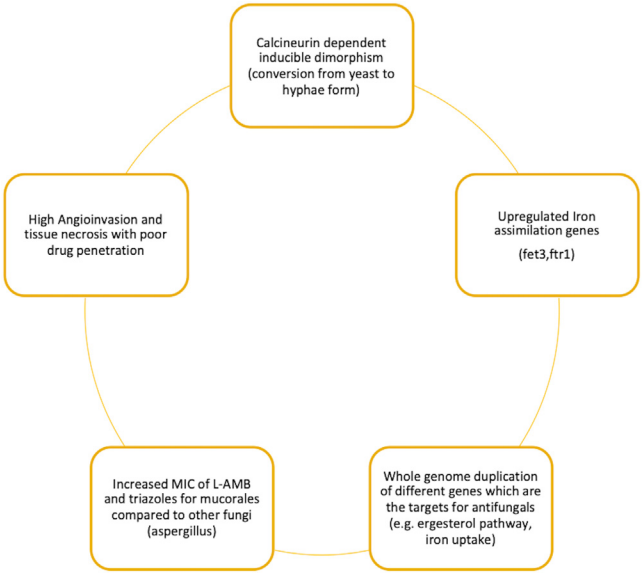

Fig. 1. Virulence factors and possible pathways attributing to the inadequate antifungal response in Mucorales infection.

survival with AMB and caspofungin in mice with diabetic ketoacidosis and disseminated R. arrhizus infection [7]. Similarly, Reed et al. also showed the superiority of polyene and caspofungin combination over monotherapy [8]. In their report, the combination therapy was independently associated with improved outcomes (OR = 10.9) in proven rhino-orbital cerebral disease (mostly diabetic patients). Though the sample size was small (forty-one patients) and non-randomised, still results were encouraging. Of note, A larger retrospective study (101 patients) that compared the caspofungin-AMB combination with monotherapy did not show an improved outcome (90 days survival, 54% vs 59%, p = 0.67) [9]. However, Most of the study population was neutropenic and had prior exposure to voriconazole which may be one of the confounding factors. Another retrospective report was published by Kyvernitakis et al. in 2016; a propensity analysis failed to demonstrate the superiority of this combination in hematological transplant recipients (HCT) (mortality rate 43% vs 41%, p = 0.85) [10]. Furthermore, one recent report showed poor outcomes in HCT recipients treated with various antifungal combinations in non-Aspergillus fungal infections (mostly Mucorales) [35]. Heterogenicity of results in these studies could be due to the study population (studies that failed to show benefit with combination antifungals had a majority of patients with hematological malignancy and transplant recipients with pulmonary disease, which is associated with a poor outcome).

Amphotericin with Triazoles (Posaconazole and Isavuconazole)

Based on preclinical and retrospective data, the initial evidence of posaconazole use in Mucorales infection is encouraging. Still, no prospective data is available to give an insight into combination therapy. However, this combination has more potential than the earlier discussed combination (caspofungin with L-AMB). Both drugs (AMB and triazoles) individually have shown promising efficacy and act on two different targets of Mucorales (Triazoles act on lanosterol 14α-demethylase). Furthermore, the combination is at least expected to reduce the therapeutic doses so that adverse events can be minimized. Some recent animal models have described the synergistic role of triazoles and polyenes [36,37]. The first murine model that described a better survival rate with low dose AMB (0.3 mg/kg/day) and posaconazole combination compared to monotherapy in immunosuppressed mice with disseminated disease. Of note, this combination was effective in only one out of two Mucorales strains [36]. A recent in vitro study by Gebremariam et al. further consolidated this finding [37]. A combination of isavuconazole and L-AMB showed a better survival (80% vs 50%) compared to monotherapy in a neutropenic mouse with pulmonary disease [37]. In the recent COVID-19 pandemic, few retrospective clinical data (diabetic patients with ROCM disease) have strengthened the aforementioned findings [38,39]. According to a recent retrospective study by Patel et al., no survival benefit was observed with the use of posaconazole/L-AMB combination (diabetic patient with ROCM disease) [40]. Again, despite the recent surge of cases and increasing use of combination antifungals, no randomized data is available. In addition, most COVID-19 cases had ROCM disease due to uncontrolled diabetes and inadvertent use of steroids. This poses another important challenge of extrapolating these findings into other groups, e.g., pulmonary or disseminated mucormycosis.

Triple therapy (Amphotericin B, Azoles, and Echinocandin)

Data is sparse regarding the use of triple combination therapy in mucormycosis. To conduct such study, there will be a need for a larger population which can compare triple combination with dual antifungal therapy or monotherapy. Few clinical studies (retrospective data) described the use of triple combinations; none of these reports showed improved outcomes with a triple combination [9,10,35,41,42]. Though the sample size was very small, and no prospective data is available to date. We have summarised the available evidence from the literature on combination antifungals in table 2.

Off-label agents for adjunct therapy

There are few antifungals/formulations in pipelines (oral amphotericin, ibrexafungerp, and fosmanogepix) for the treatment of invasive mold infections. However, all these drugs are in the phase 2/3 stage, and further data is required before their use in Mucorales infections as a combination therapy [43]. Apart from antifungal combinations, some other therapeutic agents have been incorporated with AMB with mixed results over the years. The host iron acquisition is the key to the pathogenesis of Mucorales. Thus, iron deprivation as an adjunct to antifungals looks attractive to achieve better results. In 2007, Ibrahim et al. first discussed the fungicidal activity of deferasirox against Mucorales in an in vitro study [44]. Unfortunately, this result did not reflect in vivo studies. DEFEAT Mucor study (randomized trial) showed increased 90 days mortality with a deferasirox/L-AMB combination [45]. We need further evidence from the larger studies to validate these preliminary data. COVID-19 and uncontrolled diabetes are the iron excess states, and the surge of mucormycosis in the current pandemic can provide the opportunity to conduct such trials. However, the drug shortage (AMB) could be an important limitation during the current pandemic [46]. These subsets of patients (COVID-19 with diabetes) might be benefitted most from iron chelators compared to patients with neutropenic/hematological malignancy.

Another interesting hypothesis is the use of statins with antifungals. 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase enzyme is a key enzyme responsible for ergosterol synthesis. A murine model was proposed by Bellanger et al., which described the utility of statins in attenuating the virulence of *R. arrhizus* [47]. Statin can attenuate the GRP 78 overexpression and ER stress [48], which are the important triggers for mucormycosis, especially in cases with uncontrolled diabetes and COVID-19. Mucormycosis is an angioinvasive disease, and tissue hypoxia and poor wound healing remain a critical issue even post-debridement. HBOT (hyperbaric oxygen therapy) can augment tissue healing by increasing vascularity and neoangiogenesis. In addition, it has antifungal properties like reducing spore germination and fungal growth and promoting oxidative burst and phagocytosis [49]. A retrospective case study described the adjunctive role of HBOT in mucormycosis with diabetes (94% survival) [49]. Calcineurin inhibitors and probiotics are the other adjunctive therapy used in various studies that showed a synergistic role along with L-AMB [50,51].

While discussing the role of combination therapy, it is imperative to differentiate it as a primary treatment or part of refractory disease management (as a salvage therapy). The outcome may differ in both settings, which should be explored in further studies.

Table 2
Various antifungal combinations, clinical characteristics, and outcomes in Mucormycosis.

Author	Study Model	Antifungal combination (doses)	Risk Factors	Organ involved	Outcome
Spellberg et al. (2005)	Murine Model	ABLC/Caspofungin (ABLC=5 mg/kg) (Caspofungin =1 mg/kg)	Diabetes	Disseminated (brain/kidney)	improve outcome with combination therapy (p = 0.05)
Reed et al. (2008)	Retrospective Clinical study (41 patients)	AMB/Caspofungin (AMB=1 mg/kg) (L-AMB= 5 mg/kg)	Diabetes	ROCM	improve outcome with combination therapy (OR=10.9)
Rodriguez et al. (2008)	Murine model	AMB/Posaconazole (AMB=0.3 mg/kg) (Posaconazole=40 mg/kg)	immunosuppressed	Disseminated	Improved survival
Abidi et al. (2014)	Retrospective Clinical study (101 patients)	L-AMB/Posaconazole L-AMB/Caspofungin	Hematological malignancy/transplant recipients	ROCM (51%). Lung (21%)	No effect on survival
Kyvernitakis et al. (2016)	Retrospective clinical study (106 patients)	L-AMB/Posaconazole or L-AMB/Caspofungin or L-AMB/Caspofungin/Posaconazole	Hematological Malignancy	Lung	No effect
Patel et al. (2020)	Retrospective clinical study (465 patients)	L-AMB/Posaconazole	Diabetes (74%)	ROCM (67%) Lung (13%)	No survival benefit
Gebremariam et al. (2021)	Murine model	L-AMB/Isavuconazole	Neutropenia	Lung	improved survival with combination Therapy
Glampedakis et al. (2021)	Retrospective clinical study (9 patients)	ABM/Echinocandin or AMB/Posaconazole or AMB/Posa/Echinocandin	Post HCT	Lung	Decreased survival with combination therapy
Patel et al. (2021)	Retrospective clinical study (287 patients)	L-AMB/Posaconazole	COVID-19 Diabetes	ROCM (85%) Lung(7%)	No survival benefit

ABLC= Amphotericin B lipid complex, L-AMB= Liposomal Amphotericin B, ROCM= Rhino-orbito-cerebral mucormycosis, OR= Odds Ratio.

Conclusion

The widespread dissemination of mucormycosis cases in recent times is alarming and needs immediate attention from worldwide clinical researchers. The clinical spectrum of mucormycosis is constantly evolving and stretching the health care facilities due to the high mortality and lack of new armamentarium in antifungals. With the recent surge of Mucorales infections, this is the appropriate time to explore the possibilities of combination antifungals. Newer shreds of evidence are also emerging favouring the combination therapy in invasive mucormycosis, which combines echinocandins, newer triazoles and iron chelators with AMB. There is an abundance of preclinical data, which needs to be validated by larger prospective studies. However, the feasibility of such trials in mucormycosis management remains to be seen.

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Declaration of Competing Interest

The authors have no conflict of interest.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.mycmed.2022.101332.

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