Current Combination Antifungal Therapies – The Need for Safer, More Effective Options

a report by

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Despite considerable progress in the past few years, the morbidity and mortality of invasive fungal infections are still unacceptably high.[1] There is a need for antifungal drugs with new mechanisms of action that have a broad spectrum of activity (including resistant pathogens) and can be administered both intravenously and orally. Moreover, agents with these characteristics plus a favourable safety profile and few drug interactions would be attractive to evaluate as components of combination therapy regimens for infections that are difficult to treat. Predicting the clinical outcome of a systemic mycosis is often a difficult task, especially when microbiological resistance is one of the factors contributing to therapeutic failure. Some of these factors are host-related (e.g. immune status, site and severity of infection and compliance to therapy), while others are associated with drug characteristics (e.g. dosage, type of compound (fungistatic/fungicidal), pharmacokinetic properties and drug–drug interactions). This article focuses on specific clinical scenarios where combination therapy is being evaluated.

What Makes an Antifungal Drug Effective?

The features of an ideal antifungal include a broad spectrum of activity, maximum bioavailability, extensive distribution and penetration throughout all body compartments and cidality. With the exception of amphotericin B (AMB), most antifungal drugs are fungistatic. AMB acts by concentration-dependent killing of fungal cells and by prolonged persistent effects. The goal of the standard dosing regimen is to maximise tissue concentrations. The major pharmacokinetic parameters of AUC/minimum inhibitory concentration (MIC) and peak/MIC correlate with efficacy. Azole antifungals act by time-dependent killing and prolonged persistent effects (duration related to AUC). The goal of the dosing regimen is to optimise the amount of the drug. The AUC/MIC is the major parameter correlating with efficacy. In summary, the development of new antifungal drugs has benefited from our understanding of pharmacokinetic–pharmacodynamic (PK–PD) parameters.

New Monotherapy Strategies

The broad spectrum of conventional AMB has prompted new strategies to improve antifungal treatment beyond our current understanding and experience of empirical and pre-emptive treatment. One approach has been to assess higher dosing of liposomal AMB. Indeed, the pharmacodynamic properties, pre-clinical data from animal models and the response rates of patients who received doses greater than 3mg/kg/day suggested that liposomal AM could improve outcomes and survival. However, a randomised comparative trial did not demonstrate a greater benefit of a 10mg/kg/day dose over the lower dose.[2] In the setting of invasive aspergillosis (IA), it is clear that new strategies are needed. Recent experiences indicate that the echinocandin caspofungin is not appropriate for either preventing breakthrough IA in an empirical usage setting[3] or, as a recent European Organisation for Research and Treatment of Cancer (EORTC) study indicates, as first-line treatment for IA.[4] These studies emphasise the importance of a fungicidal agent for this disease.

Combination Strategies – Linking Evidence with Experience

The benefits of drug combinations have been demonstrated largely in infectious diseases such as HIV infection and tuberculosis. Generally, the principle of combination therapy is to combine drugs with differing pharmacological targets. It has been predicted that the simultaneous inhibition of fungal cell wall biosynthesis and disruption of cell wall integrity may result in synergistic interaction against pathogenic yeasts and filamentous fungi. During the past decade, new antifungals have been developed that give the clinician an opportunity to choose from a broader arsenal of drugs for the treatment of invasive fungal infections. Moreover, there is now a greater possibility of combining agents with different modes of action to achieve an additive or synergistic action for the most severely ill patients. Clinical studies are difficult to perform with this group of patients, and there are insufficient and somewhat conflicting data from *in vitro* studies, animal models and clinical reports on the relative efficacy of different antifungal combinations. Clearly, before combining agents, it

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is important to understand the mode of action of the individual compounds and at least rule out antagonistic effects that could be deleterious to patients. The optimal combination would possibly be drugs with different pharmacokinetics and sites of action.

Efficacy of Combination Therapy in Invasive Aspergillosis

As effective monotherapy is available and reasonably effective for candidosis, recent interest in combination therapy has focused on IA. Studies on antifungal combinations *in vitro* against *Aspergillus* species and in animal models of aspergillosis have produced variable results, depending on the study methodology and strains evaluated. Studies of combination therapy with an echinocandin and AMB or an echinocandin and an azole in patients with IA suggest a potential benefit associated with combination therapy. An exhaustive review of combination therapy for IA is provided by Steinbach.[5]

The first prospective open-label non-comparative study of combination therapy with an echinocandin was recently reported.[6] In this study, 53 patients received caspofungin in combination with voriconazole, itraconazole or AMB as salvage therapy for IA. A favourable response was observed in 29 of 53 patients (55%) overall: eight of 16 (50%) who received caspofungin plus a polyene and 21 of 37 (57%) who received caspofungin plus a triazole.

Although using combinations of agents with different mechanisms of action is appealing, the available data are difficult to interpret. Unanswered questions include which combination would be optimal, what end-point is appropriate, how much benefit would have to be seen to justify the adoption of combination therapy and which populations are likely to benefit. Despite the frequent clinical use of antifungal combination therapy for primary or salvage therapy of invasive fungal infection in many centres, to date no randomised study comparing monotherapy with combination therapy has been performed.

Candida Biofilms and Combination Therapy

Various types of candidosis are associated with the formation of biofilms.[7] Increased antifungal resistance of *Candida*, when displaying the biofilm mode of growth, was first demonstrated by Hawser and Douglas.[8] *Candida* biofilms were 30–2,000 times as resistant as planktonic cells to various antifungal agents, including AMB, fluconazole, itraconazole and ketoconazole.[8] Subsequent researchers have confirmed these observations.[7] Resistance to fluconazole has been explored with particular interest. *In vitro* fluconazole resistance of *Candida* biofilms can range from 250 to 400 times that of planktonic *Candida*.[7] *In vivo*, *Candida* biofilms also display increased fluconazole resistance: *Candida* biofilms had an MIC for fluconazole that was 128 times higher than that of planktonic *Candida*.[7] This property has clinical implications. In addition, limited studies have suggested that caspofungin and liposomal AMB are particularly effective against *Candida* biofilms.[7] Several studies have explored the potential antagonism between AMB and azole agents in the treatment of candidosis. There is a theoretical concern that azole agents will interfere with the activity of AMB. Various experimental studies have confirmed this.[9] For a number of years there has been concern that when fluconazole is administered before AMB (a typical prophylaxis or sequential therapy scenario) there is reduced activity of the AMB. For example, in a rabbit model in which AMB was administered after treatment with fluconazole there was slower clearance of *Candida* from infected tissues than after treatment with AMB alone.[10]

A recently published study is illustrative of the mode of action of different antifungal classes in a biofilm model. Kuhn and colleagues examined the antifungal susceptibilities of *C. albicans* and *C. parapsilosis* biofilms grown on a bioprosthetic model.[11] In addition to conventional agents, the authors determined whether new antifungal agents (triazoles, AMB lipid formulations and echinocandins) have activity against *Candida* biofilms. The effects of pre-incubation of *C. albicans* cells with subinhibitory concentrations (sub-MICs) of drugs to see whether they could modify subsequent biofilm formation were also explored. Susceptibility testing of fluconazole, nystatin, chlorhexidine, terbenafine, AMB and the triazoles voriconazole and ravuconazole revealed resistance in all *Candida* isolates examined when grown as biofilms compared with planktonic forms. In contrast, lipid formulations of AMB (liposomal AMB and AMB lipid complex [ABLC]) and echinocandins (caspofungin and micafungin) showed activity against *Candida* biofilms. Pre-incubation of *C. albicans* cells with sub-MIC levels of any of the above-mentioned antifungals decreased the ability of cells to subsequently form biofilms. Microscopic analysis of planktonic and biofilm-associated blastospores showed that treatment with voriconazole, caspofungin and AMB lipid formulations resulted in morphological alterations. In conclusion, the data reveal that *Candida* biofilms show unique susceptibilities to echinocandins and the lipid formulations of AMB.

The mode of action of antifungal combinations on *Candida* biofilms has also been studied.[12] Planktonic and sessile antifungal susceptibilities of clinical isolates from invasive infections were determined for AMB deoxycholate, caspofungin and voriconazole. Sessile susceptibilities were determined for the combination of caspofungin and voriconazole. Planktonic MIC90 values and sessile MIC90 (sMIC90) values were 0.25 and 2, 0.06 and >256 and 0.5 and 2mg/l for amphotericin, voriconazole and caspofungin, respectively. The sMIC90 of the combination of caspofungin and voriconazole against sessile isolates was 0.5/2mg/l. The source of *C. albicans* clinical isolates did not appear to affect *in vitro* biofilm formation. Susceptibility to antifungal agents decreased when *C. albicans* was associated with biofilm formation, and the combination of caspofungin and voriconazole did not appear to provide enhanced activity compared with caspofungin alone.

A Look into the Future

Recent studies in experimental models of IA have suggested that the association between AMB and caspofungin decreased tissue infection and increased survival.[13,14] Case reports and retrospective studies have indicated that the combination of caspofungin with a lipid formulation of AMB or an azole may be beneficial as salvage therapy. Other studies have suggested that a combination of voriconazole and caspofungin might result in improved survival in patients with IA.[15] More clinical studies are obviously needed, and the following study may serve as a model for future trials.[16]

Patients with proven or probable IA were randomised in a prospective, open pilot study to receive either a combination of liposomal AMB at 3mg/kg daily and caspofungin 70mg on day one followed by 50mg daily thereafter or monotherapy with high-dose liposomal AMB (10mg/kg/day). Thirty patients (21 men and nine women) with haematological malignancies were analysed (15 patients in each arm). The median duration of treatment was 18 days for the combination group and 17 days for the high-dose monotherapy group. At the end of treatment, there were significantly more favourable overall

responses (partial or complete responses; p=0.028) in the combination group (10 of 15 patients; 67%) compared with the high-dose monotherapy group (four of 15 patients; 27%). Survival rates at 12 weeks after inclusion were 100 and 80%, respectively. Infusion-related reactions occurred in three patients in the high-dose monotherapy group. A two-fold increase in serum creatinine occurred in four of 17 patients (23%) who received high-dose monotherapy and one of 15 patients (7%) who received combination therapy; hypokalaemia <3mmol/l occurred in three and two patients, respectively.

Another recent clinical report of combination therapy illustrates the potential of using an echinocandin with fluconazole. Puius and Scully reported a case of pericarditis due to *C. albicans* in a heart transplant patient, which presented as tamponade approximately three weeks post-transplant in the absence of evidence of sternal osteomyelitis.[17] Pericarditis due to *Candida* species is a rare clinical entity, associated with thoracic surgery and immunosuppression. The patient was treated with pericardiocentesis and a combination of caspofungin and fluconazole, but ultimately required the explantation of retained epicardial leads and the creation of a pericardial window. This case illustrates that *Candida* must be considered in the differential diagnosis in post-transplant pericarditis, and that foreign body removal is key in helping to resolve such infections. This case also demonstrates the first use of caspofungin with fluconazole to treat *Candida* pericarditis.

The treatment of refractory oral candidosis in oral cancer patients is a further example of the need for new approaches to therapy. On oral surfaces, mixed-species biofilms are common and a true treatment challenge.[18] Success with a systemic antifungal alone would require the availability of high concentrations of the antifungal in the saliva, unlikely to take place in a dry mouth. Combining the systemic antifungal with topical synergistic antifungals, typically nystatin and/or AMB, is often required for a complete response.

What has been termed the 'third age of antimicrobial therapy' is the combination of efungumab (Mycograb), which is an antibody to heat shock protein 90 (hsp90) and lipid formulations of AMB.[19–21] Efungumab has been developed as a major recombinant antibody fragment against hsp90 that incorporates the dominant paratope found in patients who have recovered from invasive candidosis. Efungumab and other hsp90 inhibitors, such as geldanamycin, have an antifungal effect *in vitro* and, in the case of efungumab, in an animal model of fungal infection. In a randomised, double-blind study in patients with invasive candidosis, the combination of efungumab and lipid-associated AMB was superior to montherapy with lipid-associated AMB, demonstrating a higher clinical response rate (86 versus 52%; p<0.001), a higher mycological response rate (89 versus 54%; p<0.001), a faster rate of culture-confirmed clearance (hazard ratio 2.3; 95% confidence interval 1.4–3.8; p=0.001) and less *Candida*-attributable mortality (18 versus 4%; p=0.025).[21] In the setting of invasive candidosis, the echinocandin casofungin appears to be an effective treatment, but the response rate of 50–53% underlines the need to improve clinical outcome. Resistance to caspofungin has increasingly been reported and hsp90 is implicated in this process.[22] This raises the question as to whether the addition of efungumab to caspofungin therapy will increase sensitivity and lead to an improvement in overall treatment success rates. This hypothesis has been examined at a pre-clinical level. In experimental models of invasive candidosis and *in vitro* tests there was a significant reduction in MICs and positive biopsies in mice on combination therapy, demonstrating that efungumab increased the sensitivity of *Candida* to caspofungin.[23]

General Conclusions

Single-drug therapy, either as first-line or salvage treatment, rarely obtains response rates greater than 50–60%. Combination therapy is an attractive concept for treating invasive mycoses. Optimal combination regimens remain unclear. However, given its broad spectrum, encompassing difficult to treat patients, it is believed that liposomal AMB is the drug of choice for invasive mycosis combination therapy. It is envisaged that in the future the echinocandins anidulafungin and micafungin, as well as azole antifungals in development, will be combined with liposomal AMB to explore their potential in treating systemic fungal infections. ■

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