Combination Therapy for Invasive Mycoses:
Evaluation of Past Clinical Trial Designs

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Background. Despite the availability of new antifungals, single-agent therapy frequently falls short of high cure rates. Combination therapy offers potentially higher cure rates, especially for resistant organisms. In vitro studies and experimental animal models have provided conflicting data.

Methods. Retrospective, randomized, controlled clinical studies were reviewed.

Results. Results indicate a clear advantage for polyene and flucytosine combination therapy in cryptococcal meningitis and a possible advantage for combination amphotericin B and fluconazole for candidemia. Unfortunately, the few studies published have been flawed by design problems that have compromised the determination of outcome. Study review allows investigators the opportunity to design future studies to ensure optimal evaluation of efficacy.

Conclusion. Combination antifungal therapy is advantageous in cryptococcal diseases and may have a role in the treatment of invasive candidiasis. The greatest potential exists for combination therapy against aspergillosis and resistant fungi in patients with refractory mycotic disease who experience failure of monotherapy. Although there has been considerable progress in the treatment of invasive mycoses, there remain numerous unresolved clinical issues, and therapeutic outcome remains far below our expectations in many specific areas. In particular, there is scant information as to the utility of combination therapy with antifungals for treatment of mycotic disease. Moreover, the clinician is faced with confusing, contradictory in vitro data as well as a paucity of animal studies to guide the use of antifungal combinations in clinical practice.

Combination therapy with antimicrobials is used for a number of reasons (table 1) [1]. The first goal is to achieve synergy to produce enhanced clinical activity and thus a faster and superior curative effect. Ideally, a drug combination would enhance fungicidal activity. The second advantage of combination therapy is in the context of initial empirical selection of antifungal drugs. A second agent could enhance and broaden the spectrum of activity of the agents at a time that critical information regarding the pathogen is still not available to the clinician. In this scenario, synergy is not at issue—the broadened spectrum of activity simply decreases the likelihood that the patient will not receive an appropriate antifungal agent during those critical first hours of therapy. Once the microbial agent is identified, combination therapy might no longer be necessary. A third benefit of combination therapy is the potential to reduce the emergence of resistant mutant fungal pathogens. Emergence of resistance is well known in such clinical entities as tuberculosis and Pseudomonas infections. However, there is scant information available as to how often susceptibility of the pathogen alters during an invasive fungal infection. Currently, with rare exceptions, acquisition of resistance during therapy, even when long term, has not emerged as a major problem. Clearly, additional studies will be needed to evaluate this concept. The fourth potential advantage of combination therapy would be to reduce the toxicity of the antifungal agents used, provided that the combination allows reduced dosing of the individual agents. Under these circumstances, one might anticipate better tolerability of each agent. It is also clear that the combination therapy can, at times, increase toxicity, because of the additional potential for each agent to induce its own toxicity as well as the potential for synergistic toxicity. The latter is exemplified when amphotericin B and flucytosine are used in the
presence of progressive amphotericin B–induced renal insufficiency, which in turn enhances flucytosine toxicity.

Combination therapy for invasive fungal infections is most commonly considered a therapeutic option in cases of treatment failure with monotherapy. Of the various antifungal combinations available, prospective clinical trial data are available for only 3 combinations: amphotericin B plus flucytosine for cryptococcal disease, amphotericin B plus azoles for candidemia, and azoles plus flucytosine for mucosal candidiasis. At this juncture, except for the efficacy of amphotericin B plus flucytosine against cryptococcal infection, none of the 3 combinations has been proven clearly superior to monotherapy. Accordingly, the clinician is faced with a lack of information from controlled studies involving antifungal combinations. On the other hand, there exist multiple case reports describing patients for whom combination therapy may have been helpful under uncontrolled circumstances, and usually in the context of failure of monotherapy. In particular, we are likely to see antifungal combinations. On the other hand, there exist multiple case reports describing patients for whom combination therapy may have been helpful under uncontrolled circumstances, and usually in the context of failure of monotherapy. In particular, we are likely to see combination therapy used as an alternative to monotherapy in dealing with multiresistant fungi such as Scedosporium, Scopulariopsis, and Fusarium species.

**AMPHOTERICIN B PLUS FLUCYTOSINE COMBINATION THERAPY FOR CRYPTOCOCCAL MENINGITIS**

The superiority of combination antifungal therapy over monotherapy in patients without AIDS who have cryptococcal meningitis was first evident in 1979 [2]. A combination of amphotericin B and 5-flucytosine over a 6-week period was associated with a more rapid clinical response than that of 10 weeks of monotherapy. In addition, a higher cure rate was obtained (>70% vs. 50%). The superior activity of this combination was, in part, a function of enhanced in vitro activity as well as the superior penetration of flucytosine. Not long after this critical antifungal combination trial, a dramatic increase in cryptococcal meningitis was seen in patients as a result of the AIDS epidemic [3]. It soon became apparent that in patients with AIDS, cryptococcal disease proceeds more aggressively, is more difficult to eradicate, and is accompanied by an extremely high relapse rate [3]. Early studies with amphotericin B or fluconazole monotherapy failed to show high cure rates, particularly during the acute phase of treatment, achieving only a 34%-40% success rate [4]. Subsequently, a retrospective study by White et al. [5] showed that amphotericin B at a dose of 1.0 mg/kg/day in combination with 5-flucytosine at a dose of 100 mg/kg/day achieved a cure rate of 78% of patients with AIDS. Accordingly, a large, prospective multicenter study was undertaken to use this antifungal combination in the treatment of patients with AIDS. In this study, treatment with amphotericin B at a dose of 0.7 mg/kg/day and 5-flucytosine at 100 mg/kg/day was compared with monotherapy with amphotericin B at 0.7 mg/kg/day (step 1) [6]. At 2 weeks, there was a strong trend toward increased sterilization of the CSF with the combination therapy (60% vs. 51%; \( P = .06 \)). Not only did the higher-dose amphotericin B (0.7 mg/kg/day) and 5-flucytosine (25 mg/kg q6h) combination achieve a more rapid and enhanced rate of sterilization of CSF, but the overall 2-week mortality was diminished. At 2 weeks, the study provided a second step at which patients, regardless of their initial randomization, were randomized to receive either itraconazole or fluconazole, at 400 mg/day for 8 weeks. Fluconazole was associated with a higher rate of CSF sterilization than was itraconazole, but there was no difference in clinical outcome [6]. The overall clinical outcome did not differ significantly, and the overall mortality was remarkably reduced by both step 1 and step 2 therapy, achieving an overall mortality of 5.5% in the first 2 weeks for step 1 and 3.9% over the next 8 weeks. A third randomization occurred at 10 weeks, with patients randomized to either fluconazole or itraconazole, at 200 mg/day for the next 12 months [7]. The study was stopped by the data safety monitoring board when 13 (23%) of 57 patients who were randomized to receive itraconazole experienced a culture-positive relapse of cryptococcal meningitis, compared with only 2 (4%) of 51 patients receiving fluconazole (\( P = .006 \)). Most important to note is that the factor most closely associated with relapse during the maintenance phase was for the patient not to have received 5-flucytosine during the initial 2 weeks of therapy (relative risk, 5.88; \( P = .04 \)).

This was the first of the modern prospective, randomized controlled studies evaluating combination therapy. The overall message was that there was no clinical in vivo antagonism when azoles were used after amphotericin B and that amphotericin B and flucytosine provided an initial advantage over monotherapy. Nevertheless, this study is not without criticism [6, 7]. The first problem was that the 3 steps involved in this prospective study increased the complexity of analysis, which compromised the evaluation of the benefits of 5-flucytosine. Although the initial patient population was divided into 2 study arms, by step 2 there were 4 arms and by step 3 there were many more study arms, making it more difficult to evaluate the beneficial effects of combination therapy. Adding to the complexity was the fact that 25% of the patients initially randomized to treatment had dropped out and were not available at the 10-week evaluation point, and, therefore, there was con-

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**Table 1. Reasons for the use of combination therapy for mycoses.**

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<th>Reason</th>
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<td>Synergistic activity—i.e., enhanced activity</td>
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<td>Enhanced/broadened spectrum of activity</td>
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<td>Reduced toxicity due to lowered dosage of individual agents</td>
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<td>Decreased emergence of resistance</td>
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siderable loss of information, particularly with regard to CSF sterilization as an end point. Another major criticism of this study was the fact that 25% of the patients randomized at step 3 into the maintenance phase of the study had not participated in step 1 or step 2 of the initial study. Accordingly, this substantially increased the variables and heterogeneity of the patient population treated.

Another problem of the combination study was the failure to address a frequently asked clinical question regarding the importance of achieving CSF sterilization at the end of the acute phase of therapy [8]. Although sterilization of CSF on day 14 correlated with sterilization at the end of 10 weeks, there had been too many changes in treatment regimens, which precluded a clear answer to this question. Accordingly, there is still no clear conclusion as to whether initial amphotericin B, with or without flucytosine, should be continued until CSF sterilization has been achieved. It is not current clinical practice to require CSF sterilization before a switch to consolidation fluconazole therapy. This point emphasizes the important principle that drug studies should be driven by the needs of clinicians, with regard not only to selection of optimal therapy but also to dose and duration of therapy. The relative failures of the study also serve to emphasize that prospective drug studies should not attempt to answer too many questions within a single study and that the introduction of multiple steps and multiple variables ultimately serves only to weaken the conclusions when the study is analyzed. There can be no question that use of a combination of drugs adds to the complexity of determining the contribution of individual drugs and that the study design should keep confounding variables to a minimum so as to confidently answer the most pressing clinical questions.

COMBINATION ANTIFUNGAL THERAPY FOR CANDIDEMIA

Clinicians have approached the use of combination therapy with azoles and amphotericin B with extreme caution. This is because of a mass of confusing in vitro data, predominantly based on checkerboard and other methodologies. Similarly, some studies using time-kill methods have found antagonism between amphotericin B and fluconazole toward *Candida*. In 1995, Sugar et al. [9] demonstrated the absence of antagonism in vivo and, in fact, an additive effect in animals (murine candidiasis). This study formed the foundation for the randomized controlled study that was undertaken by the National Institute of Allergy and Infectious Diseases Mycoses Study Group [10]. In this study, monotherapy with fluconazole at 800 mg/day together with placebo was compared with fluconazole at 800 mg/day plus amphotericin B at 0.7 mg/kg/day. The rationale for this combination therapy was based on several premises. In an earlier study of monotherapy that compared fluconazole with amphotericin B, persistent candidemia still occurred in 12%–14% of patients [11]. In addition, given the changing epidemiology of candidemia in nonneutropenic patients, clinicians were faced with an increased incidence of candidemia due to species other than *Candida albicans* [12, 13]. These species demonstrate higher fluconazole MICs and, not infrequently, higher amphotericin B MICs [14]. It had become the clinical practice for clinicians to use initial empirical combination therapy with amphotericin B and fluconazole, at a range of doses, until the identity of the *Candida* species was revealed 24–72 h later. This practice of using *Candida* species identification to determine antifungal drug selection was becoming a standard of care. This approach, together with the knowledge that empirical combination therapy could rapidly be converted to monotherapy on the bases of the clinical response of the patient and species identification was the reason for this combination study. The end points in the combination study attempted to reduce the rates of persistent candidemia as well as to shorten duration of candidemia and possibly reduce mortality.

The use of a higher dose (800 mg) of fluconazole in both arms of the study represented a leap of faith based on a single small study in which there was some evidence to suggest that a higher dose was more effective for critically ill patients [15]. There was minimal information in the literature to support this increase, although higher doses of fluconazole were widely used by the infectious diseases community, on the basis of the proven safety record of fluconazole together with the knowledge that blood serum concentrations could thus be achieved that could inhibit those relatively resistant strains of *Candida glabrata*. In the final analysis of the study, the overall success rate was significantly higher with the combination therapy, 69% vs. 56% (P = .04), and in a Kaplan-Meier time-to-failure analysis, the success rate by study day 30 was 57% in the fluconazole arm and 69% in the combination arm (P = .08), which also reflected a trend toward success with the combination therapy [10]. Failure to clear the bloodstream occurred in 17% of patients receiving monotherapy and in 6% of patients receiving both fluconazole and amphotericin B (P = .02). The importance of this study was provision of data in a large, well-controlled study of nonneutropenic patients that showed that this combination of antifungal therapies for candidemia is not antagonistic. Moreover, there was a strong trend toward greater success and more rapid clearance of the bloodstream. Not surprisingly, the patients on the amphotericin B arm had a higher frequency of toxicity; however, because of the short duration of amphotericin B administration, few patients were dropped from the study because of toxicity.

The study indicated that combination therapy for candidemia is clearly a short-term option for early therapy, during which combination drugs would be offered not only because of a broader spectrum but also because of enhanced efficacy.
The study also indicated that clinical trials evaluating combination regimens could be designed in vivo and that the additive or synergistic effect of 2 drugs could be measured in vivo. At the same time, all of the existing weaknesses of monotherapy studies remained pertinent, particularly with regard to the optimal end points of trials of antifungals [16].

The most widely used end point remains resolution of candidemia, together with resolution of accompanying symptoms. This end point, unfortunately, remains clouded by the numerous other factors that influence resolution of candidemia, including failure to remove a catheter or to drain a responsible abscess. Other major variables in candidemia are the various, infrequently clarified, portals of entry and risk factors. The problems of resolution of clinical signs and symptoms include the fact that manifestations are entirely nonspecific, and the frequent coexistence of concomitant bacterial infections and associated pathology further confound outcome. Duration of candidemia, per se, as an end point is similarly complicated by the various factors mentioned above. Another potential end point suggested is the need to alter antifungal therapy [16]. This highly subjective decision is a very controversial issue and is unlikely to be acceptable as an end point in the near future. Overall mortality is an end point that is always measured in studies of monotherapy and combination therapy. Mortality or survival remains complicated by our continued inability to adequately measure underlying disease and by the failure of the APACHE score to necessarily reflect this predisposition. We may well reach a situation in which a composite score is used as a means of evaluating antifungals in candidemia studies. A major lesson from these drug combination studies is that we learn more from treatment failures than from successes, and the simpler the protocol the better. Too many steps inevitably introduce additional confounding variables that prevent us from understanding the benefits of the second antifungal agent.

CONCLUSION

Although multiple fungal combinations have been used in clinical practice, there are clinical data from only 3 studies: amphotericin B plus flucytosine, amphotericin B plus azoles, and azoles plus flucytosine. The most convincing evidence supporting combination therapy comes from the use of amphotericin B plus flucytosine against cryptococcal infections. The latest studies of combination therapy to treat candidemia indicate that combination therapy may well have a role compared with monotherapy [9]. It is likely that combination therapy, as an alternative to monotherapy, will also be used in the future treatment of multiresistant fungi, as well as in patients with refractory aspergillosis. There clearly is a need for additional prospective antifungal drug-combination studies, and there appears to be no scientific rationale to explain why these studies should not proceed without delay.

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References