Interactions of Itraconazole with Amphotericin B in the Treatment of Murine Invasive Candidiasis

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The interactions of amphotericin B and itraconazole were studied in murine invasive candidiasis. Candida albicans–infected mice were treated for 10 consecutive days, 24 h after infection. Survival was monitored over 30 days and kidney cultures were done. Mice treated with amphotericin B (0.2 mg/kg/day intraperitoneally) or itraconazole (100 mg/kg/day by oral gavage in two divided doses/day) had a 30-day survival of 20% or 40%. Concomitant administration of both drugs resulted in 100% mortality; 90% of mice treated with amphotericin B (1 mg/kg/day) survived. With the combination, 100% were dead by day 28 ($P \leq .001$ vs. amphotericin B). With sequential therapy (i.e., 5 days with one drug and then 5 days with the other), survival was inferior to that with amphotericin B alone but similar to that with itraconazole alone. Kidney culture results confirmed the antagonism of the combination compared with amphotericin B alone. In treatment of murine invasive candidiasis, the concomitant or sequential use of amphotericin B and itraconazole results in a negative interaction.

While theory predicts that azole antifungal drugs will antagonize the effects of amphotericin B in the treatment of fungal infections, it has been difficult to prove this [1]. For example, in vitro testing frequently predicts drug antagonism [2–4], yet experience with in vivo models of mycoses often show additive or synergistic effects [5–8]. Moreover, the effects of an azole on the efficacy of amphotericin B seem to be specific to the azole, and broad generalizations may not accurately reflect the interaction [1, 9, 10].

A theory to explain these drug-to-drug differences has been advanced [11]. Briefly, lipophilic azoles, such as itraconazole, are thought to block the interaction of amphotericin B at the cell membrane, the major site of action of amphotericin B, by adsorbing to the cell membrane surface. Water-soluble azoles, such as fluconazole, on the other hand, partition into the fungal cell and do not accumulate on the cell membrane, thereby allowing amphotericin B to bind to cell membrane ergosterol. Thus, the interaction is more complicated than merely inhibition of ergosterol synthesis by the azole resulting in a decrease in the major amphotericin B target in the fungal cell membrane.

In addition to these drug-specific concerns, there may be differences in the end result of such combination therapy depending on the fungus involved. For example, several different animal models of aspergillosis have been used to demonstrate antagonism of amphotericin B with azoles [7, 12, 13], yet in other animal models (e.g., cryptococcosis, trichosporonosis, and candidiasis), positive effects are noted [6, 14, 15]. Thus, both drug-specific and fungus-specific factors may dictate the type of interaction seen with combined use of polyene and azole antifungal drugs.

Despite these concerns regarding antagonistic interactions between some azoles with amphotericin B, the use of azoles concomitantly with amphotericin B or sequentially, after an initial course of amphotericin B, is commonly observed in clinical practice. Therefore, we conducted this study using a well-described murine model of invasive candidiasis to evaluate potential interactions between amphotericin B and itraconazole in the treatment of invasive candidiasis in this mouse model.

Materials and Methods

Fungi. Candida albicans 64, a well-characterized isolate in our laboratory [5, 10], was maintained in stock culture at −70°C. When needed for an experiment, blastoconidia were grown for 48 h on fresh Sabouraud dextrose agar slants. Blastocytomycida were harvested and washed twice with sterile saline, and desired concentrations were prepared in saline.

Mice. Three-week-old male ICR mice (weight, 16–20 g) were obtained from Harlan Sprague-Dawley (Indianapolis). Mice were acclimatized for at least 2 days before infection. They were fed food and water ad libitum.

Compounds. Amphotericin B with sodium deoxycholate was purchased from Sigma (St. Louis) and was stored as a stock solution of 10 mg/mL in sterile water at 4°C. Itraconazole was obtained from Janssen Pharmaceutica (Titusville, NJ) and was prepared fresh every 2–3 days. Itraconazole was suspended in polyethylene glycol (molecular weight, 200) for oral administration to mice.

Invasive candidal infection. Mice were infected with ~4 × 10$^6$ blastocytomycida by injection into the lateral tail vein. There were 10 mice/group. In experiments in which organs were cultured, an
additional 4 mice/group were included. Treatment was begun 24 h after infection and continued for 10 days. Itraconazole was given by oral gavage twice daily and amphotericin B was given by intraperitoneal injection once daily. The polyethylene glycol vehicle for itraconazole was well-tolerated in this and our previous experiments, and no clinically evident toxicity was observed. In selected experiments, 1 day after completion of therapy or on day 30, 2 mice in each group were sacrificed for culture of kidney. Organs were homogenized (Tissumizer; Tekmar, Cincinnati), and serial dilutions were plated on blood agar plates and incubated for 48 h, and then colonies were counted.

**Itraconazole serum concentrations.** One mouse was selected at random, and blood was sampled 4 h after it received itraconazole on days 2, 4, 6, 8, and 10. On day 12, an additional mouse had blood taken 24 h after the last dose of itraconazole. Serum concentrations were measured by bioassay using *Candida kefyr* as the indicator organism by an adaptation of the method used at the Fungus Testing Laboratory, San Antonio, Texas.

**Statistical analysis.** Survival was plotted as Kaplan-Meier curves, and groups were compared by log rank analysis. Comparisons of colony-forming units from kidney cultures were analyzed by *t* test using a commercially available statistical program (SPSS, Chicago). Significance was defined as *P* < .05.

**Results**

**Concomitant therapy with amphotericin B and itraconazole.**

The first experiment evaluated whether itraconazole (100 mg/kg/day in two divided doses) could enhance the effect of suboptimal doses of amphotericin B (0.2 mg/kg/day), similar to what had been observed with fluconazole [5]. As shown in figure 1, mice infected with 4 × 10⁶ cfu of *C. albicans* all died by day 21, with a median survival time (MST) of 5 days. Itraconazole afforded significantly improved survival compared with that in controls (*P* = .002), but amphotericin B alone or in combination with itraconazole did not (*P* > .05). Survival of all groups at 30 days was <50%. The combination of amphotericin B and itraconazole was associated with 100% mortality by day 23, with an MST of 17 days (*P* = .011 compared with itraconazole alone; *P* > .05 compared with amphotericin B alone). In contrast, 20% and 40% of mice treated with amphotericin B or itraconazole survived to day 30, with MSTs of 18 and 29 days, respectively. No enhanced activity of the combination compared with either drug used alone was seen.

A second experiment focused on the effects of such combination therapy when optimal doses of amphotericin B were used. In this experiment, amphotericin B at 1 mg/kg/day with or without itraconazole at 100 mg/kg/day was studied. As shown in figure 2A, all control mice had died by day 15, with an MST of 8 days. Eighty percent of mice treated with itraconazole alone also died by day 30, with an MST of 21 days (*P* = .008 compared with control). In mice treated with amphotericin B, survival to day 30 was 90%, with MSTs of >30 days. As in the first experiment, mice treated with both drugs together for 10 days had poor survival, with 100% dead by day 28 and an MST of 10 days (*P* ≤ .001 compared with amphotericin B alone; *P* = .053 compared with itraconazole alone).

In addition to the higher dose of amphotericin B used in this experiment, the same low doses of amphotericin B were also used in this experiment, and the results were identical to those presented in the first experiment (data not shown).

Kidney cultures obtained 1 day after the end of therapy showed an ~2-log decrease in colony-forming units in amphotericin B
tericin B–treated mice compared with control mice ($P < .05$ vs. all groups; figure 2B). In contrast, no decrease was seen in mice that received itraconazole alone or the combination.

**Sequential use of amphotericin B and itraconazole.** To investigate whether sequential administration of the two antifungal drugs had any interactions, mice were infected as in the previously described experiments and treated with a combination of amphotericin B and itraconazole or with 5 days of one of the drugs followed by 5 days of the other drug for a total of 10 days of therapy. As shown in figure 3A, control mice all died by day 16, with an MST of 13 days. In contrast, 90% of amphotericin B–treated mice survived until the end of the experiment on day 30. Similar to the earlier experiments, amphotericin B plus itraconazole (survival, 0; MST, 14 days) was less effective than amphotericin B alone (survival, 90%; $P < .001$) and similar in efficacy to itraconazole alone (survival, 10%; MST, 13 days; $P > .05$). Initial treatment with amphotericin B for 5 days followed by itraconazole for 5 days resulted in a delay in mortality, but after about 2 weeks into the experiment, the slope of the mortality was similar to that seen in mice treated with itraconazole alone. At 30 days, 30% of mice treated with amphotericin B followed by itraconazole survived, with an MST of 21 days. When mice were treated with itraconazole first and then with amphotericin B, all mice died by day 27 and the MST was 18 days. Thus, all possible combinations of amphotericin B and itraconazole resulted in a decrease in the efficacy seen with amphotericin B when used as a single agent for the entire course of therapy.

Kidney cultures were done on day 30 in mice surviving to that point (figure 3B). Amphotericin B alone resulted in a significant decrease in colony-forming units compared with itraconazole and amphotericin B, followed by itraconazole ($P < .05$). There were no surviving mice in the other groups, so these cultures could not be done.

Itraconazole serum concentrations were determined for 2 of 10 days of therapy. As shown in figure 3A, control mice all died by day 16, with an MST of 13 days. In contrast, 90% of amphotericin B–treated mice survived until the end of the experiment on day 30. Similar to the earlier experiments, amphotericin B plus itraconazole (survival, 0; MST, 14 days) was less effective than amphotericin B alone (survival, 90%; $P < .001$) and similar in efficacy to itraconazole alone (survival, 10%; MST, 13 days; $P > .05$). Initial treatment with amphotericin B for 5 days followed by itraconazole for 5 days resulted in a delay in mortality, but after about 2 weeks into the experiment, the slope of the mortality was similar to that seen in mice treated with itraconazole alone. At 30 days, 30% of mice treated with amphotericin B followed by itraconazole survived, with an MST of 21 days. When mice were treated with itraconazole first and then with amphotericin B, all mice died by day 27 and the MST was 18 days. Thus, all possible combinations of amphotericin B and itraconazole resulted in a decrease in the efficacy seen with amphotericin B when used as a single agent for the entire course of therapy.

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Discussion

The data presented herein demonstrate a remarkable negative interaction when amphotericin B is combined concomitantly or sequentially with itraconazole. These results are in striking contrast with those obtained in an earlier study of amphotericin B and fluconazole in the same murine model of invasive candidiasis, in which the combination of drugs provided enhanced activity [5]. This apparent antagonism of the effects of amphotericin B by itraconazole was demonstrable by measuring survival over a 30-day period, MST, and cultures of the major target organ in this model, the kidney. Thus, it is clear from this work and that of others that the outcome of combination polyene-azole therapy depends on the identity of theazole. These data extend observations obtained from different animal models of aspergillosis [7, 12, 13] and indicate that an antagonistic interaction between amphotericin B and itraconazole is now demonstrable in vivo in two different mycoses. Clearly, given the potential for using these two drugs in combination either concomitantly or in sequence in clinical medicine, there is a need to study this form of therapy in animal models of cryptococcosis, histoplasmosis, and coccidioidomycosis, as well as in other mycoses. Until the mechanisms underlying these drug interactions are better understood, there is little else to rely on when making therapeutic decisions for the treatment of fungal diseases.

As discussed by Scheven and colleagues [11, 16, 17], one mechanism for the antagonism of amphotericin B and itraconazole may be that the lipophilic itraconazole blocks the interaction of amphotericin B binding to the fungal cell membrane. This would explain differences seen with itraconazole and fluconazole in in vivo studies. Using in vitro studies to predict the outcome of such combination therapy does not seem to be reliable, given the discordance between results obtained with in vitro analysis of fluconazole–amphotericin B interactions [18, 19] and those obtained in an animal model [5]. Thus, until
better in vitro systems are developed to more accurately predict polyene-azole interactions, such combinations need to be studied in appropriate animal models of mycoses to assess their potential for positive or negative effects in clinical medicine.

Because itraconazole is well-tolerated and poses less of a problem with respect to administration of the drug than does amphotericin B, patients requiring prolonged therapy have often been treated with amphotericin B and then switched to itraconazole for the duration of their therapy [20, 21]. On the basis of the aspergillosis data reviewed above and the data reported here, such an approach may not be warranted and in fact may be detrimental. The mechanism responsible for the antagonism seen when amphotericin B is followed by itraconazole is not easily understood in terms of the Scheven hypothesis. More likely, the duration of initial amphotericin B therapy is too short and the switch to itraconazole on day 6 results in the growth of residual fungi before steady-state concentrations of itraconazole are achieved in serum. Whatever the explanation, until further information indicates otherwise, it would seem prudent to minimize the use of amphotericin B and itraconazole in combination when treating patients.

In summary, this study demonstrates profound antagonism of the antifungal effects of amphotericin B by itraconazole when the two drugs are used concomitantly or sequentially in treatment of murine invasive candidiasis. Careful consideration should be given before these two drugs are used together in the treatment of patients with candidiasis.

Acknowledgments

This is publication no. 014 from the Collaborative Medical Mycology Research Program.

References