Challenges in Designing Animal Studies To Detect Antagonism of Polyene Activity

We read with interest the recent report by Najvar et al. (3) describing the combined activity of posaconazole (POS) and amphotericin (AMB) in a murine model of invasive pulmonary aspergillosis (IPA). Unlike previous reports that have documented attenuation of AMB activity following prior ketoconazole or itraconazole exposure (2, 4, 5), Najvar et al. were unable to find any evidence of antagonism in simultaneous or sequential combination regimens when antifungal activity was assessed by quantitative lung (CFU) cultures or animal survival. Moreover, the authors’ findings were consistent between two laboratories (Schering Plough Research Institute [SPRI] and the University of Texas Health Science Center—San Antonio [UTHSCSA]), which performed the animal testing in parallel using the same animal model and methods. Taken as a whole, the authors’ findings would appear to dispel theoretical concerns of possible antagonism between POS and AMB and pave the way for future combination therapy trials in humans.

Because attenuation of AMB activity is the expected outcome if antagonism occurs between POS and AMB (6), reproducible documentation of highly effective AMB would appear to be the required starting point of any animal study designed to detect antagonism for this combination. Therefore, we were perplexed why Najvar et al. used an animal model of IPA to study POS-AMB combinations that could not reproducibly demonstrate highly effective AMB therapy. Investigators at either study site were unable to show substantial reductions in lung fungal burden (CFU) for AMB-treated animals (5 mg/kg of body weight/day) compared to control animals, and experiments performed at the SPRI found identical survival curves for AMB-treated animals and control (untreated) animals. Indeed, evidence for activity of AMB monotherapy could be found only in survival data from the UTHSCSA, where 40% of deed, evidence for activity of AMB monotherapy could be assessed by quantitative lung (CFU) cultures or animal survival. Moreover, the authors’ findings were consistent between two laboratories (Schering Plough Research Institute [SPRI] and the University of Texas Health Science Center—San Antonio [UTHSCSA]), which performed the animal testing in parallel using the same animal model and methods. Taken as a whole, the authors’ findings would appear to dispel theoretical concerns of possible antagonism between POS and AMB and pave the way for future combination therapy trials in humans.

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Prior studies demonstrating antagonism for sequentialazole-AMB combinations shared several common characteristics not found in the study of Najvar et al. (3). (i) Antagonism was demonstrated in vitro for test isolates prior to in vivo testing. (ii) Drug concentrations and/or fungistatic or fungicidal titer were documented in animals used for in vivo testing. (iii) Azole therapy was administered for >1 day before AMB therapy. (iv) AMB monotherapy prolonged survival in 60 to 90% of older mice with IA (20 to 30 g) and reduced tissue fungal burden by >1 log₁₀ CFU compared to controls. The lack of these critical features in the study by Najvar et al. may explain, in part, why the authors could not detect attenuation of AMB activity.

REFERENCES


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Authors’ Reply

We thank Dr. Lewis and Kontoyiannis for their interest and comments. The authors focus on differences between our studies and theirs regarding primarily the lack of AMB activity in our in vivo model. Their comments bring into clear focus the limitations of experimental parameters. Kontoyiannis et al. and Lewis et al. found that itraconazole antagonized AMB in vitro and in vivo (2, 3). In contrast, Najvar et al. found that AMB did not antagonize POS in vivo (4). Different questions were evaluated by the different methods.

The two study designs were not directly comparable. Najvar et al. (4) treated an established infection at 24 h with POS and with the addition of AMB at 24 (concurrent) or 48 (sequenced) h. Furthermore, two independent laboratories used 8 to 12 mice per treatment group and multiple confirmatory studies in addition to the studies shown in the paper. The large numbers of mice used in the study emphasize the statistical significance and reproducibility of the data. Lewis et al. (3) used itraconazole only prior to infection and AMB was begun 2 days after itraconazole was stopped and 24 h after infection. Both studies are limited by the methods used.

AMB has shown poor clinical efficacy against IA (5). This was recapitulated by our model design. Given the increasing use of voriconazole as primary therapy for IA (1), it is relevant to ask whether the concurrent or delayed addition of AMB adversely affects the efficacy of primary triazole therapy. The study by Najvar et al. (4) shows that POS activity is not inhibited by concurrent or later addition of AMB. Accordingly, the present study (4) may well be relevant to development of
clinical regimens for azole-polyene combination therapy of this difficult disease.

Because of the marginal efficacy of AMB in our model, we have not addressed the issue of whether prior treatment with an azole may antagonize the activity of AMB. Thank you for allowing us to clarify some important distinctions between the Najvar et al. (4) and Lewis et al. (3) studies.

REFERENCES


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