Use of Terbinafine in Mouse and Rat Models of Pneumocystis carinii Pneumonia

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Terbinafine, an allylamine used to treat onychomycosis, has been reported to be active against rat Pneumocystis carinii in vitro and in vivo. By contrast, our in vitro data showed that the 50% inhibitory concentration of terbinafine against rat P. carinii is 3.7 μg/ml, a level that cannot be clinically achieved in serum. In the present study, terbinafine administered orally at doses of 20 to 400 mg/kg/day and 50 to 250 mg/kg/day was ineffective therapy for mouse and rat models of pneumocystosis, respectively. These results emphasize the complexities of P. carinii drug testing and the need for caution before considering studies in humans.

Despite improved treatment of human immunodeficiency virus, pneumonia caused by Pneumocystis carinii remains an important clinical problem in human immunodeficiency virus patients and other immunocompromised hosts. Anti-P. carinii drugs in clinical use are hampered by toxicity, limited effectiveness, and emerging resistance (10, 17, 24). The lack of interest among pharmaceutical companies in developing new agents for P. carinii has stimulated efforts to test existing drugs marketed for other purposes for activity against the organism. Terbinafine, a member of the allylamines which has been marketed for the treatment of onychomycosis, is one of these agents. Terbinafine has activity against dermatophytes, other fungi, and trypanosomes (19).

Two studies have shown that terbinafine is active against rat and human P. carinii infection at concentrations of 300 μg/ml and 0.4 to 0.8 μg/ml, respectively, in tissue culture (3, 5). The investigators also found that terbinafine given at oral doses of 15 to 80 mg/kg/day had efficacy that was equal to or greater than that of known anti-P. carinii drugs in rats with P. carinii pneumonia (5, 6). These provocative reports led to interest in possibly using terbinafine to treat pneumocystosis in humans. Before clinical studies can be contemplated, these findings should be confirmed by other investigators using different experimental approaches. Using an ATP cytotoxicity assay to screen candidate anti-P. carinii drugs, our group found that terbinafine has a 50% inhibitory concentration of 3.7 μg/ml against rat P. carinii at 72 h (16). Although this concentration indicates moderate activity on our rating scale (7, 28), it exceeds levels of terbinafine in serum that can be achieved in patients and other immunocompromised hosts. Anti-P. carinii drugs in clinical use are hampered by toxicity, limited effectiveness, and emerging resistance (10, 17, 24). The lack of interest among pharmaceutical companies in developing new agents for P. carinii has stimulated efforts to test existing drugs marketed for other purposes for activity against the organism. Terbinafine, a member of the allylamines which has been marketed for the treatment of onychomycosis, is one of these agents. Terbinafine has activity against dermatophytes, other fungi, and trypanosomes (19).

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Adult C3H/HeN mice and Lewis rats (Charles River) were housed under barrier conditions with infected mice and rats, respectively, and administered corticosteroids to induce the development of pneumocystosis as previously described (25, 26, 27, 28). After 6 to 7 weeks of immunosuppression, when the infection reached moderate intensity, the animals were randomly divided into treatment and control groups. Terbinafine (Lamsil; Novartis), which was obtained commercially, and trimethoprim-sulfamethoxazole (TMP-SXT), the standard drug, were dissolved in 2% ethanol and administered by oral gavage once daily on a milligram-per-kilogram basis for 3 weeks; during this time the animals were continued on the immunosuppressive regimen. Control animals on steroids (C/S animals) were given a placebo or received no treatment. In our model, drug effectiveness is based on organism burden rather than survival because the animals sometimes die from causes (e.g., other opportunistic infections) other than P. carinii (25, 26, 27, 28). The animals have to receive the terbinafine for at least 7 days to be included in the data analysis, because it usually takes this long to see an effect. The right lung was homogenized and stained with a selective P. carinii cyst stain (cresol echt violet), the organisms were counted in a blinded fashion, and the data were log transformed. The limit of detection was 2.23 × 104 (log10 4.35)/lung in mice and 1.12 × 105 (log10 5.05)/lung in rats. The left lung was preserved and used for other purposes (e.g., histology) as needed. Statistical analysis for data that were normally distributed (first experiment) was performed by an analysis of variance followed by Student’s t test with the Newman-Keuls correction for multiple comparisons (GraphPad Software for Science). Analysis of data that were not normally distributed (second and third experiments) was performed by the nonparametric Kruskall-Wallis test followed by Dunn’s multiple-comparison test. The α value was set at 0.05. Drug activity was also analyzed by a scoring system ranging from no activity (<5-fold reduction in organism counts) to very marked activity (≥1,000-fold reduction) (25, 26, 27, 28).

The first experiment was performed with mice. Terbinafine was administered at doses of 20 to 150 mg/kg/day, which were similar to those used by other investigators to treat mouse systemic fungal and protozoal infections (8, 11, 15, 19, 21, 22, 23, 30) (Fig. 1A). The data showed that none of the doses of terbinafine reduced the mean log10 P. carinii cyst count significantly below the 7.49/lung seen in the C/S group. By contrast, TMP-SXT lowered the cyst count by 851-fold to undetectable...
levels ($P < 0.001$). In the second experiment, terbinafine at a dose of 400 mg/kg/day did not lower the median log$_{10}$ cyst count significantly below the 8.60/lung seen in the C/S group (Fig. 1B). However, TMP-SXT decreased the count by 2,570-fold to 5.19/lung ($P < 0.001$). The third experiment was performed with rats (Fig. 2). $P$. carinii counts in animals treated with terbinafine did not differ significantly from the median count of 8.28/lung in the C/S group. TMP-SXT lowered the median cyst count by 1,698-fold to 5.05/lung ($P < 0.001$).

The present study has shown that terbinafine is ineffective as treatment in our mouse and rat models of pneumocystosis. The study also extends our previous reports that have shown a good correlation between our in vitro and in vivo analyses of anti-$P$. carinii activity (7, 16, 25, 28, 29). By contrast, terbinafine is active in vitro against fungi that cause systemic infections but has not been effective as therapy in animal models of these infections (8, 15, 21, 22). Following oral administration, terbinafine binds to the stratum corneum, dermis-epidermis, sebum, hair, and nails, where it achieves concentrations higher than those in plasma (9, 13, 18, 20). Yet, even when terbinafine reached a concentration in the lungs of about 6 g/ml after parenteral administration, the drug was ineffective in the treatment of experimental pulmonary aspergillosis (21).

One possible explanation for the conflicting results reported here and by the investigators mentioned above (3, 5, 6) is the presence of species or strain differences in the $P$. carinii infecting the animal colonies. In addition to their terbinafine data, those investigators reported findings (the effectiveness of oral pentamidine and relative lack of efficacy of oral atovaquone and albendazole) that are at variance with studies by other workers (1, 4, 12, 25, 26, 27). In vitro studies probably offer the best opportunity to look for antimicrobial resistance, because the same isolate can be used as the test organism. Our group uses $P$. carinii f. sp. carinii (6, 13). In limited studies, we have found no differences in drug susceptibility among genetically different strains of rat $P$. carinii (8). Theoretically, introduction of a $P$. carinii isolate into naive, immunosuppressed rats by techniques such as intratracheal inoculation should provide an in vivo model to study antimicrobial susceptibility (2). However, the fact that almost all commercial rat colonies have
latent *P. carinii* infection makes it difficult to correlate drug susceptibility to a specific isolate when these animals are immunosuppressed (14).

Another possible reason for the disparate results involves differences in the metabolism or pharmacokinetics of terbinafine among rats and mice. This seems less likely, because both groups of investigators performed at least some of their experiments with rats. It is also possible that there are differences in absorption of terbinafine. Early studies that used nonclinical preparations of terbinafine in experimental infections showed that levels of the drug in serum were influenced by the solvent used (15). Both groups here used the clinical formulation of the drug that is well absorbed; although there were differences (saline versus ethanol) in diluents, these seem unlikely to account for the different results. If anything, the larger doses of terbinafine in our study should have resulted in higher levels in serum. A final possibility involves the duration of terbinafine administration. The group reporting success started the drug at about week 4 of immunosuppression and continued it for another 6 to 7 weeks (5, 6). We began the terbinafine at week 7 and continued it for 3 weeks.

In conclusion, the lack of efficacy of terbinafine against *P. carinii* in this study emphasizes the complexities of *P. carinii* drug testing and the need for the results obtained by one group to be confirmed by others before considering studies in humans.

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