Structural changes in rat *Pneumocystis carinii* surface antigens after terbinafine administration in experimental *P. carinii* pneumonia

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Terbinafine is a synthetic antifungal agent which has recently been found to be highly effective against *Pneumocystis carinii*. This study evaluated the efficacy of terbinafine on rat *P. carinii* antigenic profile and the immune response by Western blot analysis, in comparison with atovaquone and co-trimoxazole in rats with pneumocystosis. Terbinafine was shown to target two specific major antigens, particularly those of 116 and 35–40 kDa. Antibodies reactive against these moieties were found in all rats treated with atovaquone and co-trimoxazole, but not in those treated with terbinafine. These surface antigen modifications could be related to disease severity and could provide additional information for monitoring the efficacy of this treatment.

Introduction

In recent years marked molecular and biological affinities have suggested that *Pneumocystis carinii* should be classified as a fungus. Antifungal agents could thus represent potential therapeutic and prophylactic drugs against this organism.\textsuperscript{1} A number of these compounds with different mechanisms of action have been evaluated extensively, both in vitro and in vivo, and seem to be promising. To date the most important agents are two classes of (1,3)-\(\beta\)-glucan synthase inhibitors, the echinocandins and papulacandins.\textsuperscript{2} Because of the high incidence of side effects associated with standard anti-*Pneumocystis* regimens, research continues to identify other antifungal agents with anti-\*P. carinii* potential, though the sensitivity of the organism to these compounds alone or in combination is not completely established.\textsuperscript{3} Recently, an antifungal and anti-protozoal compound, the allylamine-derived terbinafine, has been shown to be very effective in vitro against *P. carinii* and in the treatment of rat *P. carinii* pneumonia (PCP). This was first discovered by Contini et al.,\textsuperscript{4} and later confirmed by other investigators.\textsuperscript{5}

Although various mechanisms of action of terbinafine have been suggested, it is not known how *P. carinii* is cleared from rat lung after treatment. In this paper we examined the anti-\*P. carinii* efficacy of terbinafine by investigating the antigenic pattern of *P. carinii* isolated from the bronchoalveolar lavage fluid (BALF) of immunosuppressed rats and by assessing the specific serological response by Western blotting in comparison with two reference anti-\*P. carinii* drugs, atovaquone and co-trimoxazole.

Materials and methods

Rat immunosuppression and drug administration

PCP was induced by immunosuppressing 180–250 g male Sprague–Dawley rats (Charles River, Como, Italy) with 25 mg/L of subcutaneous methylprednisolone acetate (Solu-medrol; U pjohn, Milan, Italy) twice weekly for 10–12 weeks, and giving a low-protein diet to enhance the immunosuppression. Tetracycline was given by gavage to minimize other opportunistic infections. The percent survival was monitored. At intervals, randomly selected sample animals were killed and parasite burdens in the lungs were determined. The intensity of PCP was graded by scanning the impression smears and assigning an infection score of 0 (no infection evident) to 4+ (heavy infection).\textsuperscript{6} PCP progressed consistently over time for each of the inoculation groups. By week 4, rats began to show signs of wasting, with marked weight loss; *P. carinii* infection
Samples were centrifuged and the supernatants were tested by ELISA. Aft
0.472) was found to be significantly different from that of the control group. N
0.001, 0.7 and 8 × 10^7, respectively, compared with 23 ± 2.1 in the atovaquone-treated group, whereas histological lung changes were absent or minimal. A significant relationship (P < 0.001, r = 0.472) was found when semiquantitative cyst counts between BALF and rat lungs were compared. This suggests that the P. carinii burden in BALF correctly reflects the organism burden in the lung.

The electrophoretic pattern of BALF preparations analysed by SDS-PAGE is presented in Figure 1. Strong bands corresponding to major P. carinii antigens and ranging in size from 30 to 116 kDa were demonstrated in BALF from control rats with PCP and, similarly, in atovaquone preparations. Neither the electrophoretic profile nor the antigen recognition pattern of BALFs from co-trimoxazole-treated rats differed substantially from that of atovaquone-treated animals (data not shown). In BALF preparations from terbinafine-treated animals, the 116 kDa protein was not stained but the bands at molecular masses of 35–40 kDa were almost absent. Bands at 97, 66 and 45 kDa were occasionally seen in normal BALF samples. In preliminary experiments, sera collected before treatment and from rats treated with atovaquone recognized two or more antigens, particularly those of 116, 97, 66 and 35–40 kDa, but none of the terbinafine-treated rats had detectable antibodies against the 35–40 kDa protein, while
Effect of terbinafine on *P. carinii* in rat

A small number (two out of 30) showed a slight reactivity against the 116 kDa protein. Although no immunoreactivity towards the 116 or 35–40 kDa band was found by probing normal rat BALF with sera from infected rats or anti-*P. carinii* antiserum, the finding of faint bands on either silver staining preparations or blots is consistent with previous studies which confirm that animals as well as healthy humans have been exposed to *P. carinii*.11

The purpose of these experiments was to investigate whether Western blotting would provide meaningful data about the immune response to *P. carinii* antigens during drug administration. The 116 kDa protein is a component of the major surface glycoprotein (MSG), which participates in the attachment of *P. carinii* to alveolar epithelial cells. The immunoreactive moiety of molecular weight 35–40 kDa is the *P. carinii* antigen most commonly found in the respiratory tract in rat and in humans and most frequently recognized by the host; it can thus serve as an important marker of *P. carinii* infection.11

Among the squalene-epoxydase inhibitors, terbinafine is the most potent agent against pathogenic fungi and is also active against protozoa. Although its mechanism of activity against *P. carinii* is not fully established, there is experimental evidence that terbinafine may target two important surface *P. carinii* antigens, the 116 and 35–40 kDa proteins. These changes may impair the organism’s structural integrity and consequently either the mechanism of host–parasite interaction or the host immune response, thus providing additional information for monitoring the efficacy of this treatment.

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**References**


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