Limited Effect of Trimethoprim-Sulfamethoxazole Prophylaxis on *Pneumocystis carinii*

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Trimethoprim-sulfamethoxazole has been proven effective in the treatment and prevention of *Pneumocystis carinii* pneumonia in lower animals and humans. How effective the drug combination is in eradicating *P. carinii* from the host is not known. The immunosuppressed rat model was used to determine whether or not trimethoprim-sulfamethoxazole effectively eradicated the organism. Animals treated with trimethoprim-sulfamethoxazole for as long as 6 weeks were then placed in individual isolator cages, immunosuppressed with prednisone for 12 weeks, and sacrificed. *P. carinii* was found in the lungs of at least 90% of the drug-treated as well as untreated control groups. The data indicate that trimethoprim-sulfamethoxazole has a limited rather than a lethal effect on *P. carinii* and that protection is afforded only during the period of trimethoprim-sulfamethoxazole administration.

Within recent years, studies have demonstrated the efficacy of trimethoprim-sulfamethoxazole in the prevention and treatment of *Pneumocystis carinii* pneumonia in both experimental animals (5) and humans (1, 2, 4). Although clinical recovery is achieved in the majority of patients with *P. carinii* pneumonia treated with trimethoprim-sulfamethoxazole, no attempt has been made to determine whether *P. carinii* is completely eradicated from the host. Second episodes of the pneumonia have occurred several months after successful treatment (3). Whether these occur as reinfections or as recurrences of the previous infection is not known.

In both animal and human investigations in which trimethoprim-sulfamethoxazole was administered prophylactically, the subjects were maintained on the drug combination daily, and observations for infection were made only during the periods of drug administration. Since trimethoprim-sulfamethoxazole provides a possible approach to prophylaxis in certain immunocompromised patients at high risk for *P. carinii* pneumonia, elucidation of the minimal time required to maintain patients on such a regimen is pertinent. Since the pneumonia is probably provoked by the activation of latent *P. carinii* organisms resulting from a previous subclinical infection (6), trimethoprim-sulfamethoxazole prophylaxis given over a short period of time might eradicate the organism, and the duration of drug administration for prophylaxis could be limited.

Experimental rats have provided an excellent animal model for the study of *P. carinii* infection, because the observations made with rats correspond precisely to those made with humans. Animals immunosuppressed with a corticosteroid and protected from bacterial infection with antibiotics will develop *P. carinii* pneumonia (5). Inoculation of the organism is not required. Previous studies have shown that in rats immunosuppressed for 12 weeks, all will develop *P. carinii* pneumonia. If trimethoprim-sulfamethoxazole is given throughout the course of prednisone immunosuppression, *P. carinii* is not found in the lungs (5). However, the absence of *P. carinii* in histological sections does not conclusively prove eradication, since only portions of the total lung tissue are examined, and the small trophozoite (thin-walled cyst) forms might not be recognized if they are not associated with cyst forms (thick-walled cysts).

The objectives of the studies described here were to determine whether or not trimethoprim-sulfamethoxazole was effective in eradicating *P. carinii* and to learn whether animals so treated were subsequently susceptible to *P. carinii* pneumonia when immunosuppressed.

MATERIALS AND METHODS

Experimental design. Groups of rats were treated with trimethoprim-sulfamethoxazole for 2, 4, or 6 weeks. On the last day of treatment, each animal was placed in an individual isolator unit and given prednisone and tetracycline orally for 3 months. After 3 months of immunosuppression, the rats were sacrificed.
and the lungs were examined for *P. carinii* pneumonitis. Administration of trimethoprim-sulfamethoxazole to the immunocompetent animal before immuno-
suppression provided an optimal opportunity for host response. Placement in isolator cages at the com-
pletion of drug therapy prevented further acquisition of *P. carinii* from the environment and trans-
mission from other animals. Thus, if trimethoprim-
sulfamethoxazole successfully eradicated the organ-
ism, no evidence of infection would be expected with corticosteroid immunosuppression. A group of control animals received no trimethoprim-sulfamethoxazole (Fig. 1).

Animals. Male Sprague-Dawley rats weighing approxi-
mately 200 g were used. Before immunosuppres-
sion, the animals were maintained in the same room in open wire-top cages, having access to water and feed ad libitum. On the last day of the 2-, 4-, or 6-week courses of trimethoprim-sulfamethoxazole administra-
tion, each animal was placed in a translucent polypro-
pylene cage with an Enviro-gard filter bonnet (Labora-
tory Products, Inc., Garfield, N.J.) and housed in a differ-
ent room from the previous quarters. Standard feed and distilled water were used. Isolators were 

Drugs. Dexamethasone sodium phosphate (Merck Stick & Dohme, West Point, Pa.) was used for im-
nunosuppression in the amount of 0.2 mg per 200 ml 
of drinking water. To prevent bacterial infection, tetr-
cycline hydrochloride (E. R. Squibb and Sons, Inc., 
Princeton, N.J.) was added to the drinking water (100 mg/200 ml). Each animal was allowed 50 ml of med-
icated drinking water per day.

Trimethoprim-sulfamethoxazole (Hoffman-La Roche, Nutley, N.J.) was administered orally in drink-
ing water. The dosage was calculated on the basis of 
10 mg per rat per day (50 mg/kg per day) of trimeth-
oprime and 50 mg/kg per day (250 mg/kg per day) of 
sulfamethoxazole.

The medicated drinking water was prepared freshly 
every 2 days. The animals regularly consumed the al-
located amounts with little daily variation. The schedule for administration of drugs is depicted in Fig. 1.

Specimens. Immediately after sacrifice, the lungs 
were removed. The right and left lower lobes were 
sectioned, and imprints were made onto microscope 
slides and stained with Giemsa and toluidine blue O 
stains. The lungs were then placed in 10% Formalin 
and sections prepared for histopathology using Go-
morii's methenamine silver nitrate stain.

Staging the extent of *P. carinii* pneumonitis was 
done with the stained sections. Stage 1 was charac-
terized by isolated cyst forms found along the alveolar 
septal wall or free in the lumen with no evidence of 
inflammatory or cellular response. Stage 2 was charac-
terized by an increase in the number of organisms 

![Fig. 1. Experimental design. Hash-marked bars indicate the period of time each group of animals was subjected to trimethoprim-sulfamethoxazole prophylaxis and immunosuppression with prednisone. Isolator refers to polypropylene cages with Enviro-
gard filters.](image-url)
Comparison of sections of the right lower lobe and the left lower lobe revealed that the extent of \textit{P. carinii} infection was precisely the same in each animal.

**DISCUSSION**

The results of the experiments described above clearly show that trimethoprim-sulfamethoxazole was ineffective in the total eradication of \textit{P. carinii} when administered for as long as 6 weeks. Since no significant differences were apparent among courses of 2, 4, and 6 weeks, it is unlikely, though not proven, that courses longer than 6 weeks would be effective. The drug combination was given to presumably immunocompetent animals at a time when host response was optimal for the clearance of organisms. Placement of animals in individual isolator cages excluded the possibility of acquisition of new organisms from the environmental air after prophylaxis used during the 12 weeks of immunosuppression. Since the food and water were not sterilized by autoclaving, we cannot exclude entirely the possibility of transmission by the oral route, although this is not a known mode of transmission for \textit{P. carinii}.

Some clinical studies in cancer patients tend to support the findings in our experiments. One child with leukemia received prophylactic trimethoprim-sulfamethoxazole for a period of 26 months with no evidence of pneumonitis, but 1 month after the dosage was inadvertently reduced in half the child developed \textit{P. carinii} pneumonitis (4). \textit{P. carinii} has been occasionally found in the lungs at autopsy of patients who were successfully treated for the pneumonitis with trimethoprim-sulfamethoxazole but succumbed to unrelated complications (1). In a study by Wolff and Baehner (7), children with acute lymphoblastic leukemia were given a “high dose” of trimethoprim-sulfamethoxazole, amounting to four times the prophylactic dose used elsewhere (4), for a period of 2 weeks during induction of initial remission. A comparable control group did not receive the antimicrobial drug. Over the mean observation time of 13 months, of the 17 patients who did not receive the drug, 4 developed \textit{P. carinii} pneumonia at days 53, 60, 70, and 71 after anticancer chemotherapy was begun. Of the 19 patients who received trimethoprim-sulfamethoxazole, 2 developed the pneumonitis at days 170 and 245 after anticancer therapy was begun. Although the possibility of acquisition of new infections could not be discounted in this study, in light of the animal experiments reported here, the speculation that the 2-week course of trimethoprim-sulfamethoxazole merely delayed the onset of disease seems warranted.

These studies with murine \textit{P. carinii} suggest that trimethoprim-sulfamethoxazole is not effective in the total eradication of \textit{P. carinii}, and the reasonable conclusion can be made that immunosuppressed patients receiving this drug for prophylaxis can only be protected during the time of its administration.

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**LITERATURE CITED**


