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# 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* pneumonitis 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* pneumonitis in both experimental animals (5) and humans (1, 2, 4). Although clinical recovery is achieved in the majority of patients with *P. carinii* pneumonitis treated with trimethoprim-sulfamethoxazole, no attempt has been made to determine whether *P. carinii* is completely eradicated from the host. Second episodes of the pneumonitis 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* pneumonitis, elucidation of the minimal time required to maintain patients on such a regimen is pertinent. Since the pneumonitis 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* pneumonitis (5). Inoculation of the organism is not required. Previous studies have shown that in rats immunosuppressed for 12 weeks, all will develop *P. carinii* pneumonitis. 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* pneumonitis 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 immunosuppression provided an optimal opportunity for host response. Placement in isolator cages at the completion of drug therapy prevented further acquisition of *P. carinii* from the environment and transmission from other animals. Thus, if trimethoprim-sulfamethoxazole successfully eradicated the organism, 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 approximately 200 g were used. Before immunosuppression, 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 administration, each animal was placed in a translucent polypropylene cage with an Enviro-gard filter bonnet (Laboratory Products, Inc., Garfield, N.J.) and housed in a different room from the previous quarters. Standard feed and distilled water were used. Isolators were entered individually for food and water supplements and medication. The animals were visually examined daily. The control animals were studied at a different place and time from the drug-treated animals. A total 40 rats were used, with 10 per group. Group A received no trimethoprim-sulfamethoxazole; group B, group C, and group D received the drug for 2, 4, and 6 weeks, respectively. Animals were sacrificed in a carbon dioxide gas chamber.

**Drugs.** Dexamethasone sodium phosphate (Merck Sharp & Dohme, West Point, Pa.) was used for immunosuppression in the amount of 0.2 mg per 200 ml of drinking water. To prevent bacterial infection, tetracycline 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 medicated drinking water per day.

Trimethoprim-sulfamethoxazole (Hoffman-La Roche, Nutley, N.J.) was administered orally in drinking water. The dosage was calculated on the basis of 10 mg per rat per day (50 mg/kg per day) of trimethoprim 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 allocated 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 Gomori's methenamine silver nitrate stain.

Staging the extent of *P. carinii* pneumonitis was done with the stained sections. Stage 1 was characterized 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 characterized by an increase in the number of organisms with minimal septal inflammatory response and minimal cellular reaction in the alveolar lumen. In stage 3, an extensive reactive and desquamative alveolitis was evident with large numbers of cysts in the alveolar lumen.

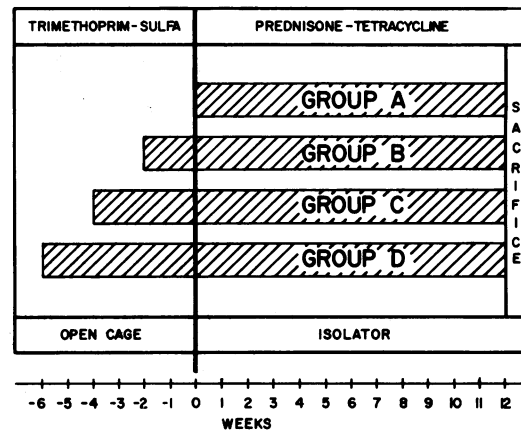


FIG. 1. *Experimental design. Hash-marked bars indicate the period of time each group of animals was subjected to trimethoprim-sulfamethoxazole prophylaxis and immunosuppression with prednisone. Isolator refers to polypropylene cages with Enviro-gard filters.*

## RESULTS

All but four of the animals survived the experiment, receiving 12 weeks of immunosuppression with prednisone. One rat in each of groups A, C, and D died on days 29, 81, and 91 of prednisone administration, respectively, and one animal in group C died before immunosuppression was begun.

At least 9 of the 10 animals in each of the four groups had pulmonary infection with *P. carinii*. The extent of involvement as estimated from tissue sections is summarized in Table 1. These data show that trimethoprim-sulfamethoxazole administered for as long as 6 weeks was ineffective in eradicating *P. carinii* and that extensive pneumonitis could be elicited with subsequent immunosuppression. Furthermore, there was no positive correlation for dose response between those treated with trimethoprim-sulfamethoxazole for 2-, 4-, or 6-week schedules.

Comparison of stained lung imprints with histological block sections of lungs showed positive correlation for the presence or absence of *P. carinii* cysts in 39 of the 40 animals. In one animal from group B, no cysts were found on the imprint, but stage 1 infection was evident on the lung sections. The imprints from this animal were extensively dense with large numbers of erythrocytes. The overly thick specimens stain heavily and are difficult to interpret.

TABLE 1. *Histopathology of lung sections*

| Category | Trimethoprim-sulfamethoxazole (week) | No. of animals | No. with *P. carinii* pneumonitis | | | |
|---|---|---|---|---|---|---|
| | | | None | Stage 1 | Stage 2 | Stage 3 |
| Group A | None | 10 | 1[a] | 0 | 0 | 9 |
| Group B | 2 | 10 | 1 | 3 | 3 | 3 |
| Group C | 4 | 10 | 1[b] | 0 | 1 | 8 |
| Group D | 6 | 10 | 0 | 1 | 3 | 6 |

[a] Early death (day 29).
[b] Died before immunosuppression.

Comparison of sections of the right lower lobe and the left lower lobe revealed that the extent of *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 *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 *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 *P. carinii* pneumonitis (4). *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 *P. carinii* pneumonitis 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 *P. carinii* suggest that trimethoprim-sulfamethoxazole is not effective in the total eradication of *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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