Inhibitors of Folic Acid Synthesis in the Treatment of Experimental *Pneumocystis carinii* Pneumonia

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Inhibitors of folic acid synthesis were compared alone and in different combinations in the therapy of pneumocystosis in immunosuppressed rats. Sulfonamides (sulfamethoxazole, sulfadiazine, and sulfadoxine) and sulfones (dapsone) used alone were very active against *Pneumocystis carinii*, as judged by histologic examination of the lungs and by organism quantitation. Improved efficacy could not be demonstrated by the addition of an inhibitor of dihydrofolate reductase to the regimen. Dihydrofolate reductase inhibitors (trimethoprim, diaveridine, and pyrimethamine) used alone were ineffective against *P. carinii*. All drugs were well tolerated except pyrimethamine, which caused bone marrow depression; folinic acid ameliorated this adverse reaction but did not interfere with *P. carinii* treatment. These data have potential clinical implications but need to be interpreted with caution and in light of other systems of *P. carinii* drug evaluation.

Although *Pneumocystis carinii* has long been recognized as an important opportunistic pulmonary pathogen (36), the organism has presented new clinical challenges in patients with the acquired immunodeficiency syndrome (AIDS). Pneumocystosis in AIDS is characterized by subtle presentation, slow response to therapy, frequent relapse, and a high rate of adverse drug reactions (10, 12, 23, 34). Inhibitors of folic acid synthesis have been a major form of *P. carinii* treatment. Attention has focused on the use of an inhibitor of dihydrofolate reductase (DHFR) (primarily trimethoprim) in combination with a sulfonamide (mainly sulfamethoxazole) (6, 13, 14, 21, 33, 40). Other DHFR inhibitors used in the treatment of clinical and experimental pneumocystosis have included pyrimethamine, tetroxoprim, pitrexim, and trimetrexate; sulfonamides and sulfones have included sulfadiazine, sulfadoxine, sulfamonomethoxine, dapsone, and sulfonylbisformanilide (1, 7, 9, 15–18, 20, 24–26, 29, 30, 39, 41, 42, 43).

Little is known about the relative efficacy and toxicity of the different folic acid inhibitors in the treatment of *P. carinii*, yet such information is important in developing new forms of therapy. In vitro approaches to drug development have involved analysis of the effects of antimicrobial agents on *P. carinii* growth in tissue culture or the uptake of dyes or radiolabeled compounds by nonreplicating organisms or by enzyme preparations (1, 2, 4, 27, 28). In vivo studies, which have been much more widely used, have been based on an animal model in which rats immunosuppressed with corticosteroids spontaneously develop pneumocystosis with histologic features identical to those of the disease in humans; drugs which are active against rat *P. carinii* have usually shown activity against human *P. carinii* (9, 14, 15). We have developed histologic and quantitative techniques to evaluate the extent of *P. carinii* pneumonia in immunosuppressed rats and to monitor the efficacy of therapy (3, 4, 5, 19, 37). In the present report, we have used these procedures to compare folic acid inhibitors, alone and in various combinations, in the treatment of experimental pneumocystosis.

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MATERIALS AND METHODS

Experimental design. The basic animal protocol has been described in detail in our previous studies (19, 37). Adult male Sprague-Dawley rats obtained from Harlan Industries, Madison, Wis., weighing about 250 g, were used in the study. After a period of acclimation in a conventional colony room, the animals were administered an immunosuppressive regimen of 25 mg of cortisone acetate (Cortone; Merck Sharp & Dohme, West Point, Pa.) injected subcutaneously twice weekly or 4 mg of methylprednisolone acetate (Depo-Medrol; The Upjohn Co., Kalamazoo, Mich.) injected subcutaneously once weekly, a low (8%) protein diet (Bioserv, Frenchtown, N.J.), and tetracycline powder (Polyotic; American Cyanamid, Wayne, N.J.) (1 mg/ml) in the drinking water to induce pneumocystosis. The rats were weighed regularly, and a few animals were sacrificed periodically to monitor the development of the disease. After 5 to 6.5 weeks, when *P. carinii* infection had reached moderate intensity, the animals were randomly divided into different treatment groups of about 16 rats each. Antimicrobial agents to be tested were administered for 3 weeks, during which time all rats remained on the immunosuppressive regimen. Control rats on this steroid immunosuppressive protocol received no therapy; pilot studies revealed no differences in the extent of *P. carinii* pneumonia among these animals or among rats administered a placebo (solution used to deliver the drugs). Also included as controls were normal rats which ate a regular diet, drank plain tap water, and received no medications. At the end of the treatment period, the rats were sacrificed by an overdose of halothane anesthesia.

Drugs. The following compounds were generously provided by Edmund Wise, Burroughs Wellcome Co., Research Triangle Park, N.C.: trimethoprim, diaveridine, pyrimethamine, sulfamethoxazole, and sulfadiazine. Diaveridine is a DHFR inhibitor similar to trimethoprim which has been used in the treatment of avian coccidiosis (32). Sulfadoxine was kindly provided by Peter Shorter, Hoffmann-LaRoche Inc., Nutley, N.J. Dapsone was obtained as a gift from David Jacobus, Jacobus Pharmaceutical Co., Inc., Princeton, N.J.

Folinic acid was purchased as leucovorin calcium from Lederle Laboratories, Pearl River, N.Y.

The drugs were prepared as a liquid suspension and administered on a milligram-per-kilogram basis as a single daily dose by oral gavage. The dose was based on the average weight of the rats (about 150 g) at the beginning of treatment and remained the same over the 3-week course of therapy. In some experiments, the dose of pyrimethamine had to be reduced because of bone marrow depression; 1 mg of folinic acid was injected subcutaneously each day in an attempt to prevent this adverse effect. The hematologic effects of pyrimethamine were monitored by performing complete blood counts on peripheral blood of rats receiving the drug and comparing the results with data obtained in animals not receiving the drug.

Evaluation of *P. carinii* therapy. Therapy was complicated by the fact that the rats, which were heavily immunosuppressed and chronically debilitated, were very susceptible to other opportunistic infections and to the toxic effects of drugs; animals frequently died before completion of treatment for *P. carinii*. Therefore, assessment of drug efficacy was based on the extent of pneumocystosis in the lungs rather than on animal survival (19). *P. carinii* infection in the drug-treated rats was compared with that in controls in the same experiment; when a compound was evaluated in more than one experiment, the control groups were pooled. Our preliminary studies suggested that 10 days was sufficient to observe a therapeutic response; unless otherwise stated, only rats which received ≥10 days of anti-*P. carinii* treatment were included in the data analysis.

Assessment of *P. carinii* pneumonia in the lungs was based on histologic and quantitative procedures previously reported in detail (3, 4, 5, 19, 37). Briefly, at death or time of sacrifice, the left lung of each animal was removed, infused with a 4% formaldehyde solution until fully expanded, and fixed. Three horizontal sections of the lung were stained with hematoxylin and eosin, which provided a general view of the pulmonary architecture, and Grocott methenamine silver, which selectively stained the cell walls of *P. carinii* cysts. The lung sections were coded (blinded) and read. The following scoring system was used to evaluate *P. carinii* pneumonia based on the extent of alveolar involvement: 0, no infection; 0.5, minimal infection (<1% alveoli involved); 1+, light infection (1 to 25% alveoli involved); 2+, moderate infection (25 to 50% alveoli involved); 3+, severe infection (50 to 75% alveoli involved); 4+, very severe infection (>75% alveoli involved).

The right lung of each animal was removed, weighed, homogenized in a Stomacher (Tekmar, Inc., Cincinnati, Ohio), and used for quantitation studies. Freshly prepared samples of the homogenate were air dried and stained with cresyl echt violet, which selectively stains the cell walls of *P. carinii* cysts, and Diff Quik (American Scientific Products, McGaw Park, Ill.), which stains the nuclei of cysts, trophozoites, and intermediate forms. The lower limit of detection by this system is 1.47×10^5 organisms per lung. Specimens were read in a coded manner.

Our previous study demonstrated that the histologic scoring system and cyst and nuclei counts provided comparable assessment of drug therapy of *P. carinii* pneumonia (19). In the present report, lung specimens of all animals were examined histologically, whereas the quantitation studies were performed on about six rats from each treatment group. Since the changes in cyst and nuclei counts after therapy were similar, only the cyst data have been presented in detail here; nuclei counts have been included on a selective basis to illustrate the changes resulting from therapy.

RESULTS

Sulfamethoxazole. Data obtained with sulfamethoxazole and DHFR inhibitors are presented in Fig. 1. The standard
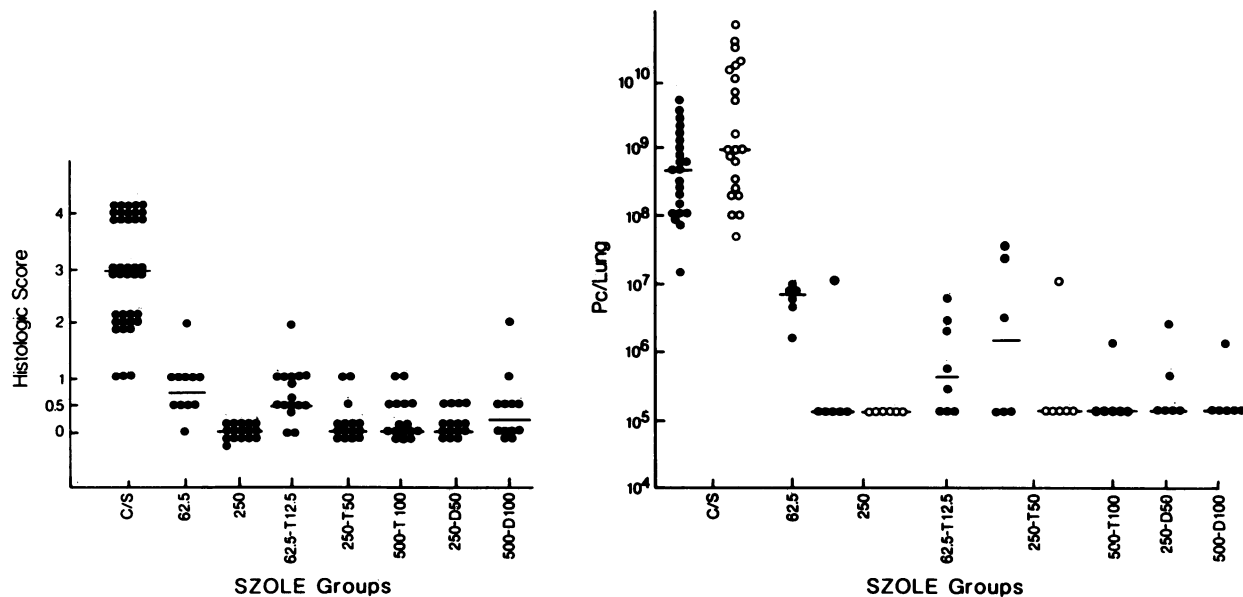

FIG. 1. Treatment of pneumocystosis with sulfamethoxazole alone and in combination with trimethoprim or diaveridine, as assessed by histologic score (left) and quantitation (right) of *P. carinii* cysts (●) and nuclei (○). The following regimens were used: sulfamethoxazole (62.5 mg and 250 mg); sulfamethoxazole (62.5 mg)-trimethoprim (12.5 mg); sulfamethoxazole (250 mg)-trimethoprim (50 mg); sulfamethoxazole (500 mg)-trimethoprim (100 mg); sulfamethoxazole (250 mg)-diaveridine (50 mg); and sulfamethoxazole (500 mg)-diaveridine (100 mg). All doses were expressed as milligrams per kilogram per day. Horizontal bars represent median values. Abbreviations: SZOLE, sulfamethoxazole; T, trimethoprim; D, diaveridine; C/S, control steroid group.
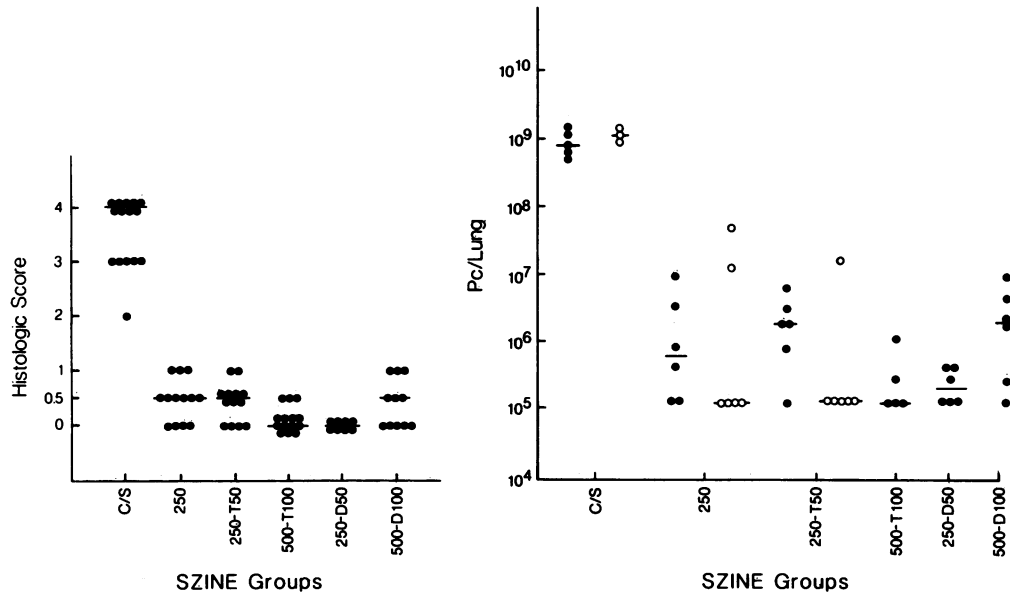
FIG. 2. Treatment of pneumocystosis with sulfadiazine alone and in combination with trimethoprim or diaveridine, as assessed by histologic score (left) and quantitation (right) of *P. carinii* cysts (●) and nuclei (O). The following regimens were used: sulfadiazine (250 mg); sulfadiazine (250 mg)-trimethoprim (50 mg); sulfadiazine (500 mg)-trimethoprim (100 mg); sulfadiazine (250 mg)-diaveridine (50 mg); and sulfadiazine (500 mg)-diaveridine (100 mg). All doses were expressed as mg/kg per day. Horizontal bars represent median values. Abbreviations: SZINE, sulfadiazine; see the legend to Fig. 1 for other abbreviations.

regimen of sulfamethoxazole at 250 mg/kg per day and trimethoprim at 50 mg/kg per day used by other investigators in the treatment of experimental pneumocystosis in rats (14, 21) was highly effective in our model. The median histologic score fell from 3+ in the control steroid group to 0 in the treated group; *P. carinii* cyst and nuclei counts declined from 5.09×10^8 and 9.11×10^8 per lung, respectively, to 1.64 $\times 10^6$ and $\leq 1.47 \times 10^5$ per lung, respectively. Similar results were found when the same dose of sulfamethoxazole was used alone or combined with diaveridine at 50 mg/kg per day or when these drugs were used in higher dose regimens. In an effort to demonstrate drug synergy, sulfamethoxazole was tested at the low dose of 62.5 mg/kg per day alone and in combination with trimethoprim at 12.5 mg/kg per day. The median histologic score and cyst count in the sulfamethoxazole-treated rats were 0.75+ and 6.91×10^6 cysts per lung, respectively, compared with 0.5+ and 4.41×10^5 cysts per lung, respectively, in the sulfamethoxazole-trimethoprim-treated rats, suggesting that trimethoprim had only a slight effect on therapeutic activity. The sulfamethoxazole regimens were well tolerated, with all rat groups having a median duration of therapy of 20 to 21 days.

Sulfadiazine. Sulfadiazine was very active in the treatment of rat pneumocystis (Fig. 2). When used alone in the dose of 250 mg/kg per day or combined with diaveridine or trimethoprim in a dose of 50 mg/kg per day, the drug resulted in a fall in the median histologic score from 4+ in the steroid controls to 0 to 0.5+ in the treated groups. Median cyst counts declined from 8.03×10^8 cysts per lung to 6.62×10^5 cysts per lung in the sulfadiazine-treated rats and 1.91×10^6 cysts per lung in the sulfadiazine-trimethoprim-treated rats, respectively, and similar changes occurred with the nuclei counts. Comparable therapeutic efficacy was found when sulfadiazine was used with diaveridine at 50 mg/kg per day or when higher drug dose combinations of these agents were used. Sulfadiazine caused no detectable adverse reactions in the rats.

Sulfadoxine. The activity of sulfadoxine against rat *P. carinii* was similar to that of sulfamethoxazole or sulfadiazine (Fig. 3). The median histologic score fell from 4+ in the steroid control group to 0 in the group treated with sulfadoxine at 250 mg/kg per day alone; median cyst and nuclei counts declined from 7.51×10^8 and 3.86×10^9 per lung, respectively, to 9.54×10^5 and $\leq 1.47 \times 10^5$ per lung, respectively. The addition of trimethoprim or diaveridine in a dose of 50 mg/kg per day to the regimen did not enhance therapeutic efficacy. Sulfadoxine was well tolerated.

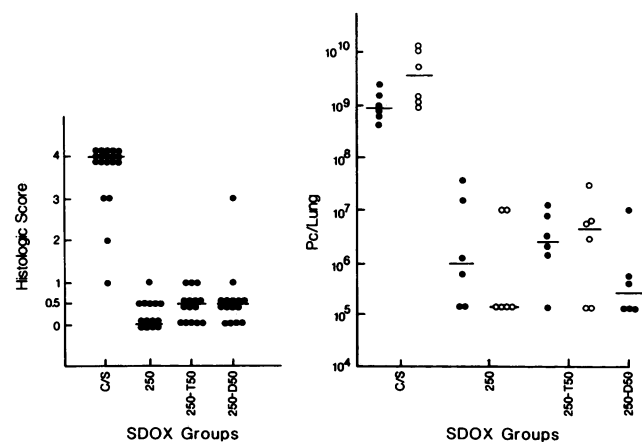**Dapsone.** Dapsone was tested at doses similar to those

FIG. 3. Treatment of pneumocystosis with sulfadoxine alone and in combination with trimethoprim or diaveridine, as assessed by histologic score (left) or quantitation (right) of *P. carinii* cysts (●) or nuclei (O). The following regimens were used: sulfadoxine (250 mg); sulfadoxine (250 mg)-trimethoprim (50 mg); and sulfadoxine (250 mg)-diaveridine (50 mg). All doses were expressed as mg/kg per day. Horizontal bars represent median values. Abbreviations: SDOX, sulfadoxine; see the legend to Fig. 1 for other abbreviations.
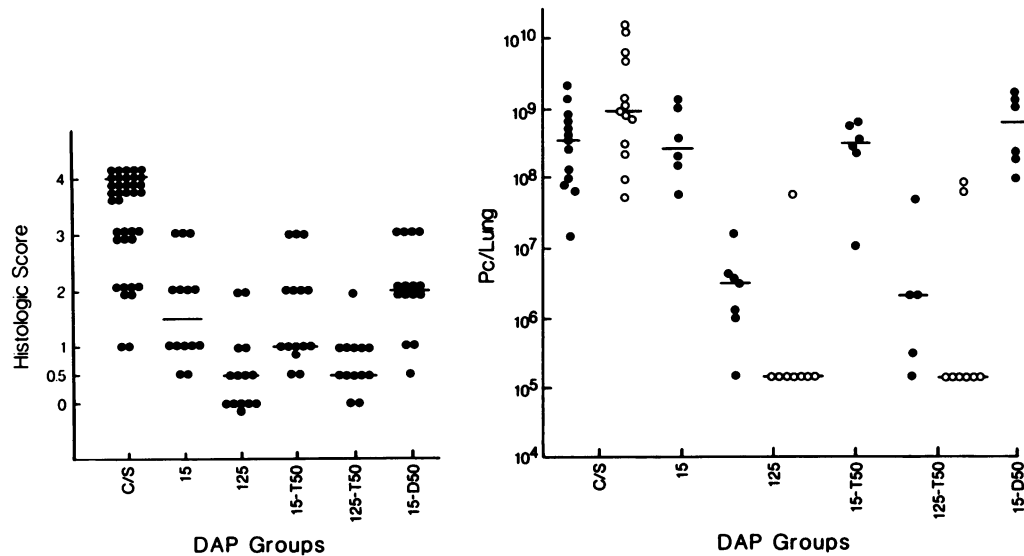
FIG. 4. Treatment of pneumocystosis with dapsone alone and in combination with trimethoprim or diaveridine, as assessed by histologic score (left) and quantitation (right) of *P. carinii* cysts (●) or nuclei (○). The following regimens were used: dapsone (15 mg); dapsone (125 mg); dapsone (15 mg)-trimethoprim (50 mg); dapsone (125 mg)-trimethoprim (50 mg); and dapsone (15 mg)-diaveridine (50 mg). All doses were expressed as mg/kg per day. Horizontal bars represent median values. Abbreviations: DAP, dapsone; see the legend to Fig. 1 for other abbreviations.

used by other workers (15) (Fig. 4). Rats administered dapsone at 15 mg/kg per day had a median histologic score of 1.5+, compared with a score of 4+ in the steroid controls; however, there was little difference between these groups on the basis of cyst counts (median value of 2.62×10^8 in the dapsone-treated rats versus 3.00×10^8 in the control steroid group, respectively). The addition of trimethoprim or diaveridine in a dose of 50 mg/kg per day to the regimen did not improve therapeutic efficacy, as judged by histologic score or organism quantitation. Dapsone administered at the higher dose of 125 mg/kg per day was more active against *P. carinii*. Rats treated with the drug alone had a median histologic score of 0.5+ and median cyst and nuclei counts of 3.23×10^6 and $\leq 1.47 \times 10^5$ per lung, respectively. Trimethoprim added at a dose of 50 mg/kg per day did not enhance treatment efficacy. Dapsone did not cause adverse reactions in the animals.

DHFR inhibitors. The DHFR inhibitors used alone showed little activity against *P. carinii* (Fig. 5). Rats administered trimethoprim in the high dose of 100 mg/kg per day had a median histologic score of 3+, a cyst count of 4.58×10^8 cysts per lung, and a nuclei count of 7.27×10^8 per lung, compared with a score of 4+, a cyst count of 6.63×10^8 cysts per lung, and a nuclei count of 3.90×10^9 per lung in the control steroid group. Similar results were noted in rats treated with diaveridine in a dose of 100 mg/kg per day. Both drugs were well tolerated.

Initial studies with pyrimethamine were plagued by serious bone marrow toxicity. The drug was administered at a dose of 18.75 mg/kg per day alone or in doses of 18.75 gradually reduced to 6 mg/kg per day combined with sulfamethoxazole at 250 mg/kg per day. The animals experienced severe leukopenia (peripheral granulocyte counts were frequently <500), and many died within a short time after receiving the drug. The data presented in Fig. 5 were obtained from rats which had received ≥ 8 days therapy. Rats administered pyrimethamine alone had a median duration of therapy of 9 days; although their median histologic score was 2+, their median cyst count (1.34×10^8 cysts per

lung) was similar to that of the control steroid group. Rats given various doses of pyrimethamine combined with sulfamethoxazole for a median duration of 12 days displayed a response to therapy with a median histologic score of 1+ and cyst count of 7.34×10^5 cysts per lung.

Further studies with pyrimethamine. Studies were then performed with lower doses of pyrimethamine and folinic acid in an attempt to ameliorate the bone marrow depression (Fig. 6 and 7). One approach involved administering pyrimethamine in a dose of 3 mg/kg per day alone or in combination with a sulfonamide. The other approach involved administering pyrimethamine in a dose of 9 mg/kg per day and folinic acid at 1 mg per day alone or in combination with a sulfonamide; however, because of ongoing hematologic problems, the pyrimethamine dose was reduced to 6 mg/kg per day. Both regimens permitted the rats to survive long enough (median duration of therapy was usually 14 to 18 days) to assess drug efficacy.

Pyrimethamine administered alone in the dose of 3 mg/kg per day or in the dose of 9 mg/kg per day to 6 mg/kg per day with folinic acid at 1 mg per day showed little or no activity against *P. carinii*; the median histologic score and cyst count of both rat groups were similar to those (i.e., 3+, 2.82×10^8 cysts per lung, respectively) of the control steroid group. Pyrimethamine administered in a dose of 3 mg/kg per day combined with sulfamethoxazole at 50 mg/kg per day or sulfamethoxazole at 250 mg/kg per day or in a dose of 9 mg/kg per day to 6 mg/kg per day with folinic acid at 1 mg/day and sulfamethoxazole at 250 mg/kg per day was effective treatment; median histologic scores fell to 0 to 1+, and cyst counts declined to 8.07×10^5 to 5.29×10^6 cysts per lung. Similar results were obtained with combinations of pyrimethamine and sulfadiazine at 250 mg/kg per day and sulfadoxine at 250 mg/kg per day. Pyrimethamine administered with dapsone at 15 mg/kg per day resulted in median histologic scores of 0.5 to 1+, but cyst counts were >10^7 cysts per lung. The combination of pyrimethamine and dapsone at 125 mg/kg per day resulted in a similar histologic score (0.5+) but lower cyst counts (2.21×10^6 cysts per lung).
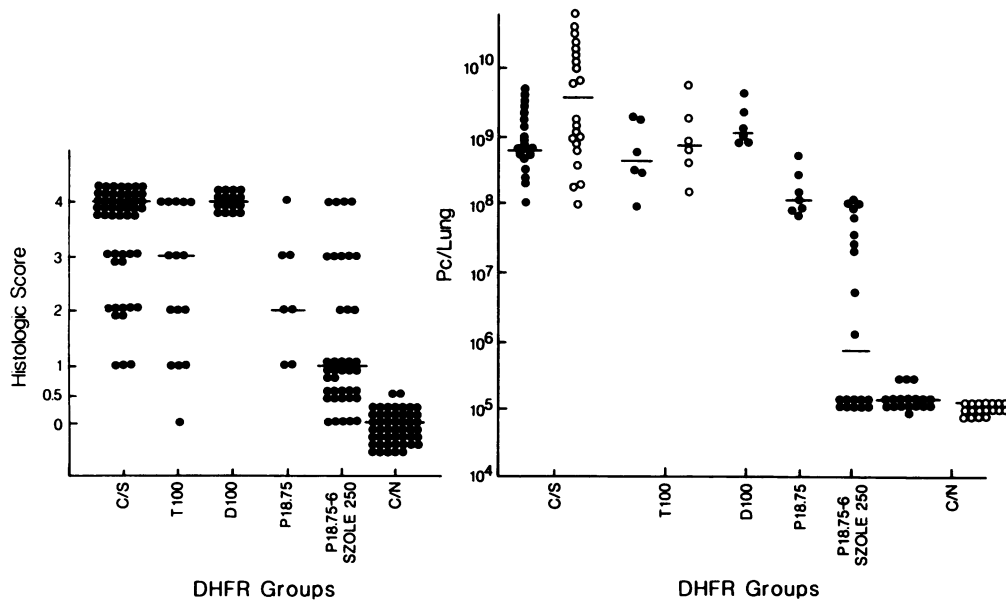
FIG. 5. Treatment of pneumocystosis with inhibitors of DHFR alone and in combination with sulfamethoxazole as assessed by histologic score (left) and quantitation (right) of *P. carinii* cysts (●) or nuclei (○). The following regimens were used: trimethoprim (100 mg); diaveridine (100 mg); pyrimethamine (18.75 mg); and pyrimethamine (18.75 mg reduced to 6 mg)-sulfamethoxazole (250 mg). All doses were expressed as mg/kg per day. Horizontal bars represent median values. Abbreviations: SZOLE, sulfamethoxazole; P, pyrimethamine; C/N, control normals; see the legend to Fig. 1 for other abbreviations.

DISCUSSION

The present study has compared different inhibitors of folic acid synthesis in the treatment of pneumocystosis. The data indicate that sulfonamides used alone are highly active against *P. carinii*. Comparable results were achieved with 250 mg/kg per day of sulfamethoxazole, sulfadiazine, or sulfadoxine, and efficacy was not enhanced by the addition of a DHFR inhibitor to the regimen. That these data are truly reflective of drug activity is suggested by the fact that dosage was controlled, and the extent of pneumocystosis was evaluated by both histologic and quantitative techniques. Sulfamethoxazole administered at a dose of 62.5 mg/kg per day was also quite active against *P. carinii*, and its effectiveness was not materially improved when combined with trimethoprim. Thus, it appears that further reduction in drug dose or alteration in experimental design will be needed to clearly demonstrate a synergistic effect between a sulfonamide and a DHFR inhibitor in this animal model.

There has been little apparent interest in studying the use of sulfonamides as single-agent therapy for clinical or experimental pneumocystosis. One previous report found that the combination of pyrimethamine and sulfadiazine was more active against *P. carinii* than was either agent used alone in limited numbers of rats (14). In contrast, there have been numerous studies of the relative efficacy of sulfonamides used alone or in combination with a ·DHFR inhibitor in experimental models of other protozoa (e.g., *Toxoplasma gondii*) (8, 11, 22, 31, 35). It has sometimes been difficult to demonstrate a synergistic effect of combination therapy for toxoplasmosis in mice, depending on such factors as the specific drug used, dose, or route of administration; in addition, differences in drug metabolism or pharmacology have made it difficult to extrapolate from data obtained in these animals to humans. In such cases, in vitro systems might provide a better measure of drug interaction (11).

In the present study, dapsone used alone exhibited dose-related activity against *P. carinii* which was not improved by

the addition of a DHFR inhibitor. Our rats were heavily immunosuppressed and malnourished, and pneumocystosis was already moderately far advanced before treatment was begun. This system was designed to mimic the situation in AIDS and might not be sensitive enough to detect subtle effects of drug interactions. In previous reports, the combination of a sulfone and trimethoprim was no more effective
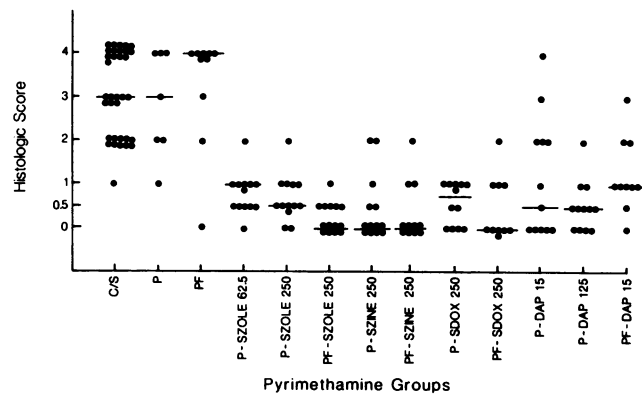

FIG. 6. Treatment of pneumocystosis with pyrimethamine and other drugs as assessed by histologic score. Pyrimethamine was administered either in the dose of 3 mg alone or in the dose of 9 mg reduced to 6 mg together with folinic acid (1 mg). These basic regimens were then combined with sulfonamides or sulfones in the following manner: Pyrimethamine-sulfamethoxazole (62.5 mg); pyrimethamine-sulfamethoxazole (250 mg); pyrimethamine-folinic acid-sulfamethoxazole (250 mg); pyrimethamine-sulfadiazine (250 mg); pyrimethamine-folinic acid-sulfadiazine (250 mg); pyrimethamine-sulfadoxine (250 mg); pyrimethamine-folinic acid-sulfadoxine (250 mg); pyrimethamine-dapsone (15 mg); pyrimethamine-dapsone (125 mg); and pyrimethamine-folinic acid-dapsone (125 mg). All drug doses except that of folinic acid are expressed as mg/kg per day. Horizontal bars represent median values. Abbreviations: P, pyrimethamine; F, folinic acid; SZOLE, sulfamethoxazole; SZINE, sulfadiazine; SDOX, sulfadoxine; DAP, dapsone.
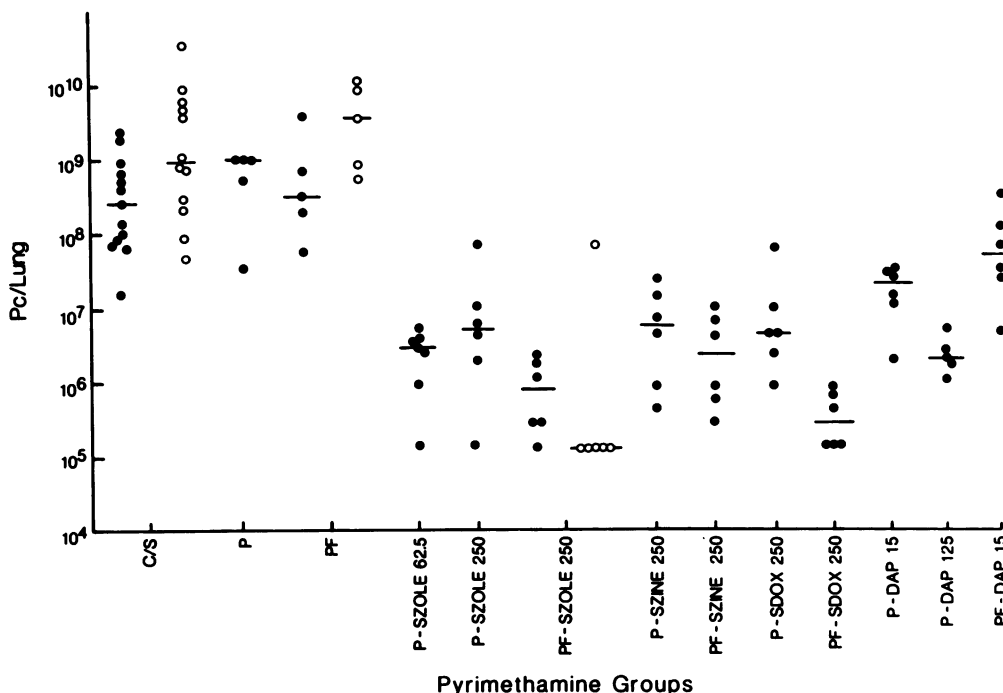
FIG. 7. Treatment of pneumocystosis with pyrimethamine and other drugs as assessed by quantitation of *P. carinii* cysts (●) and nuclei (○). The regimens in the figure are the same as those described in the legend to Fig. 6.

than the sulfone alone in therapy for well-established rat *P. carinii* pneumonia; however, with less immunosuppression, adjustment of sulfone dose, and earlier initiation of anti-*P. carinii* treatment, a synergistic effect could be demonstrated (15, 17). Uncontrolled clinical studies have also suggested that the combination of trimethoprim and dapsone is more effective anti-*P. carinii* therapy than is dapsone used alone (25).

In addition to the sulfonamides and sulfones, anti-*P. carinii* activity in the rat model has been found among selected sulfonylureas (16). It will be important to design clinical trials so that different members of these classes of drugs can be compared in the treatment of pneumocystosis under controlled conditions; individual compounds differ considerably in their pharmacology and in the type and frequency of adverse reactions. Consideration should also be given to exploring the use of these agents without a DHFR inhibitor, since some cases of rash associated with trimethoprim-sulfamethoxazole may be associated with the trimethoprim component (24).

As described in previous reports (9, 14, 21), DHFR inhibitors used alone in the present study were ineffective treatment for pneumocystosis, and there were no differences noted among these compounds in anti-*P. carinii* activity when they were combined with a sulfonamide or sulfone. Trimethoprim and diaveridine were well tolerated by the rats, but pyrimethamine caused serious bone marrow depression. This adverse effect limited not only the dose but also the length of time for which pyrimethamine could be given. Our experience with folic acid inhibitors, as well as with other agents tested so far, suggests that the duration of therapy can be an important contributor to drug efficacy in the rat model of pneumocystosis.

All drugs in the present study were administered on a milligram-per-kilogram basis as a single daily dose by oral

gavage. We feel that this method allows greater precision in drug dose than does mixing the compounds in the food or drinking water, as had been done in earlier studies. The method of drug administration has had no discernable impact on the activity of DHFR inhibitors when used alone but might have affected the results of the drug synergism experiments. Studies directly comparing methods of administration with drug pharmacokinetics and anti-*P. carinii* activity in the rat model will be necessary to answer these questions.

Recently, techniques have been developed to isolate rat *P. carinii* DHFR and to study the effects of antimicrobial drugs; a hierarchy in potency has been found among inhibitors of this enzyme with trimetrexate, an analog of methotrexate, being more potent than pyrimethamine, which is more potent than trimethoprim (1). Based on this finding, trimetrexate has shown promising activity when used alone against rat and human pneumocystosis (7, 30). Such information has important clinical implications, in light of the high frequency of toxic reactions to sulfonamides among AIDS patients (10, 23, 34, 38). Controlled clinical trials comparing DHFR inhibitors alone or in combination with other drugs for activity against human *P. carinii* are needed; yet, based on our results obtained in the rat model, there would be little impetus to perform such studies if this system were the sole means of screening anti-*P. carinii* drugs.

The dramatic rise in the number of cases of *P. carinii* pneumonia associated with the spread of AIDS has stimulated efforts to develop new forms of therapy (25). The rat model will continue to play an important role in this effort because it has usually been a reliable predictor of drug activity in humans (9, 14, 15). The present study of folic acid inhibitors emphasizes that the rat model is complex and that data obtained should be carefully interpreted and correlated with other systems of drug evaluation.

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