

Efficacy of Trimethoprim and Sulfamethoxazole in the Prevention and Treatment of *Pneumocystis carinii* Pneumonitis

WALTER T. HUGHES, PAUL C. McNABB, THOMAS D. MAKRES, AND SANDOR FELDMAN
Infectious Diseases Service, St. Jude Children's Research Hospital, and Departments of Pediatrics and Microbiology, University of Tennessee Medical Units, Memphis, Tennessee 38101

Received for publication 12 December 1973

A combination of trimethoprim and sulfamethoxazole was effective in the prevention and treatment of *Pneumocystis carinii* pneumonitis in cortisone-treated rats. Although all of 15 untreated rats died with *P. carinii* pneumonitis, none of 15 given trimethoprim-sulfamethoxazole prophylactically acquired the infection. After *P. carinii* pneumonitis was established, 9 of 14 rats recovered after treatment with trimethoprim-sulfamethoxazole compared with only 2 of 14 treated with pentamidine isethionate. Rifampin and clindamycin, separately or in combination with pentamidine, were ineffective in the prevention and treatment of *P. carinii* infection.

After World War II, epidemics of *Pneumocystis carinii* pneumonitis occurred in Central Europe but were limited to debilitated and marasmic infants. The disease was first recognized in the United States in 1955 (11) and 1956 (2), and since then has been reported with increasing frequency. The disease has occurred in the United States almost exclusively in patients with lymphoproliferative malignancies, organ transplants, congenital immunodeficiency disorders, and certain vascular disorders.

P. carinii pneumonitis expressed in the immunosuppressed host results in a mortality rate of approximately 100% if untreated (19). Pentamidine isethionate is presently considered to be the drug of choice for the treatment of this infection. The mortality rate has been reduced to as low as 32% by daily injections of pentamidine (9). However, pentamidine has a number of disadvantages: it has a relatively high therapeutic failure rate; it must be administered intramuscularly; clinical response is slow; it is not commercially available in the United States; and it is associated with undesirable toxic effects (impaired renal function, local tissue necrosis, hypoglycemia, and folic acid deficiency). Thus, search for a more effective and less toxic agent is warranted. Furthermore, it would be desirable to have a safe means of prophylaxis for use during times of high risk in immunosuppressed patients.

Pyrimethamine plus sulfonamides have been shown to have some effect against *P. carinii* (4,

13) although prophylactic usage has produced contradictory results (8, 13).

The cortisone-treated rat provides an excellent animal model for the study of *P. carinii* infection (4). The pneumonitis occurs spontaneously in 80 to 100% of the animals after prolonged immunosuppression with cortisone. The mode of transmission and details of endogenous latency of this organism are not known. Although the taxonomy of *P. carinii* has not been defined, it is most likely a protozoan. Three drugs, not previously investigated for effectiveness against *P. carinii* but with possible anti-protozoan activity, were studied in the cortisone-treated rat model.

The combination of trimethoprim and sulfamethoxazole has been effective in the treatment of patients with *P. falciparum* malaria (3). The mode of action is similar to that of pyrimethamine and sulfadiazine. Sulfamethoxazole acts by competitively inhibiting the incorporation of para-aminobenzoic acid into dihydrofolate. Trimethoprim inhibits dihydrofolate reductase, the enzyme which reduces dihydrofolic acid to tetrahydrofolic acid, an early stage in the process leading to the formation of purines and eventually deoxyribonucleic acid (DNA).

Clindamycin has been demonstrated to be effective against acute toxoplasmosis (12) and malaria (10) in animals. This drug acts as an inhibitor of protein synthesis.

Rifampin has been reported to be effective in preventing the multiplication of *Toxoplasma*

gondii in L-cell culture (16). Rifampin has a specific inhibitory action against bacterial deoxyribonucleic acid-dependent ribonucleic acid polymerase.

Prophylactic and therapeutic regimens of trimethoprim-sulfamethoxazole, clindamycin, and rifampin were investigated for effectiveness in *P. carinii* infections. The therapeutic effects of these drugs were compared with that of pentamidine. Trimethoprim-sulfamethoxazole was found to be highly effective in the prevention and treatment of this infection.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats weighing approximately 200 g were housed in the same room in wire cages with 15 animals per cage. They had access to water and feed ad libitum. *P. carinii* infection was provoked by immunosuppressive doses of cortisone according to the method of Frenkel et al. (4). Cortisone acetate (25.0 mg) was administered subcutaneously, twice weekly, until spontaneous death or sacrifice. The animals were examined at least once a day and weighed weekly.

Autopsies were done at the time of death. The thoracic and abdominal organs were examined grossly and sections were taken from the right lower lobe of the lung. Sections of the right kidney were taken from the animals receiving trimethoprim-sulfamethoxazole. The Formalin-fixed specimens were stained with hematoxylin and eosin and with Gomori methenamine-silver nitrate stains. The extent of *P. carinii* infection was rated according to the three stages described previously (9) and is summarized in Table 1. An imprint of the cut surface of each lung section was made on microscope slides, stained with toluidine blue O, and examined for cysts.

Experimental design. The animals were randomly assigned to 11 groups with 15 rats per group and treated as follows. Group 1 (control) received no cortisone and no antimicrobial agents. Group 2 (control) received cortisone throughout the experiment. Tetracycline was given to prevent bacterial infection. Group 3 (trimethoprim-sulfamethoxazole, prophylaxis) received cortisone and trimethoprim-sulfamethoxazole throughout the experiment and until death. Group 4 (trimethoprim-sulfamethoxazole, therapeutic) received cortisone throughout the experiment. Trimethoprim-sulfamethoxazole was begun in this group on the day the first animal died with *P. carinii* pneumonitis and was continued until spontaneous death or sacrifice. Group 5 (pentamidine) received cortisone throughout the experiment. Pentamidine was begun after the first animal in the group died of *P. carinii* pneumonitis and was continued for 2 weeks.

Group 6 (clindamycin, prophylaxis) received cortisone and clindamycin throughout the experiment and until death. Group 7 (clindamycin, therapeutic) received cortisone throughout the experiment. Clindamycin was started for this group on the day the index animal died with *P. carinii* pneumonitis and was continued until death. Group 8 (pentamidine and

clindamycin) received cortisone throughout the study. Clindamycin and pentamidine were administered to this group beginning on the day the index animal died with *P. carinii* pneumonitis. Pentamidine was given for 2 weeks, and clindamycin was given until death. Group 9 (rifampin, prophylaxis) received cortisone and rifampin throughout the experiment and until death. Group 10 (rifampin, therapeutic) received cortisone throughout the experiment. Rifampin was started after the first animal in the group died with *P. carinii* pneumonitis and was continued until death. Group 11 (rifampin and pentamidine) received cortisone throughout the experiment. Pentamidine and rifampin were started when the first animal in the group died with *P. carinii* pneumonitis. Pentamidine was continued for 2 weeks, and rifampin was continued until death.

Drugs. Trimethoprim-sulfamethoxazole (RO 6-2580, lot DMS 3738-6, Hoffman La Roche, Inc.) was administered orally in doses of 10 mg per rat per day (50 mg per kg per day) of trimethoprim and 50 mg per rat per day (250 mg per kg per day) of sulfamethoxazole.

Pentamidine isethionate (obtained through the National Center for Disease Control from May and Baker, Ltd., Dagenham, England) was injected subcutaneously in the dosage of 4.0 mg per rat (20 mg/kg) three times weekly over a period of 2 weeks.

Clindamycin palmitate hydrochloride (Upjohn Co.) (80 mg per rat per day; 400 mg/kg) was given orally.

Rifampin (Rimactane, Ciba Pharmaceutical Co.) was given in the dosage of 10 mg per rat per day (50 mg/kg). The drug was given orally to the animals treated prophylactically and subcutaneously in the same dose given to animals treated after infection was established.

Tetracycline hydrochloride (15 mg per rat per day; 75 mg/kg) was administered only to cortisone-treated control rats (group 2) to prevent bacterial infection.

The cause of death was determined by histological examination of the lungs with hematoxylin-eosin- and methenamine-silver nitrate-stained sections. *P. carinii* pneumonitis was considered the cause of death when the extent of infection was stage III and evidence of bacterial, fungal, and viral infections was lacking. Bacterial pneumonitis was defined as extensive areas of acute inflammatory reactions to bacterial invasion of pulmonary parenchyma. When infection from more than one organism was present and none clearly predominant, the cause of death was attributed to the mixed infection. When yeast or hyphal forms only were found in areas of extensive inflammatory response, the cause of death was considered to be of fungal origin. Pneumonitis associated with intranuclear or intracytoplasmic inclusions was categorized as viral infection. No attempt was made to identify the bacterial, fungal, or viral agents.

Orally administered drugs were prepared daily and given in a limited volume of drinking water or by gavage if the total dose was not consumed. Rifampin, clindamycin, and trimethoprim-sulfamethoxazole were given as a single daily dose.

Drug dosages were calculated according to the base-line body weights and were not changed throughout the experiment regardless of weight fluctuations.

RESULTS

All of the cortisone-treated animals had progressive weight loss, whereas incremental weight gains were observed in the control rats not receiving the immunosuppressive drug. Weight was not influenced by the type of antimicrobial agent administered (Table 1).

The control rats not receiving cortisone (group 1) remained healthy throughout the experiment, and *P. carinii* was not found in the lung sections from any of these 15 animals.

The cortisone-treated control rats (group 2) died between days 59 and 97 (median day, 78) (Fig. 1). All had extensive stage III *P. carinii* pneumonitis (Table 1).

P. carinii was not found in any of the lung sections from the animals treated prophylactically with trimethoprim-sulfamethoxazole (Table 1).

Of the 14 animals treated with trimethoprim-sulfamethoxazole after death of the index animal from *P. carinii* pneumonitis, five died with some evidence of *P. carinii* infection (Table 1). Two of the animals died within 2 days after initiation of therapy and had stage III infection, one died after 12 days of treatment with bacterial pneumonia but had stage I *P. carinii* infection, and two animals died on days 13 and 16, respectively, with stage II *P. carinii* pneumonitis (Fig. 1). Of the nine animals without *P. carinii* infection, spontaneous deaths occurred on days 28, 31, 115, 117 in four animals, respectively, and the five animals surviving on day 124 were sacrificed.

All animals readily accepted trimethoprim-sulfamethoxazole supplied in drinking water. There were no apparent adverse or toxic effects. Folic acid levels were not determined. Histological examination of the kidneys of animals who received trimethoprim-sulfamethoxazole for as long as 124 days showed no evidence of abnormality.

The first death from *P. carinii* pneumonitis occurred on day 68 of cortisone treatment in group 5. Of the 14 rats treated for 2 weeks with pentamidine, deaths occurred from day 70 to 124 (median day, 77) (Fig. 2). Extensive necrotic areas occurred in the skin, subcutaneous tissue, and muscles where injections were given. Extremity paresis and impairment of mobility were obvious in these animals.

The groups receiving clindamycin or rifampin prophylactically, or clindamycin, clindamycin plus pentamidine, rifampin, or rifampin plus

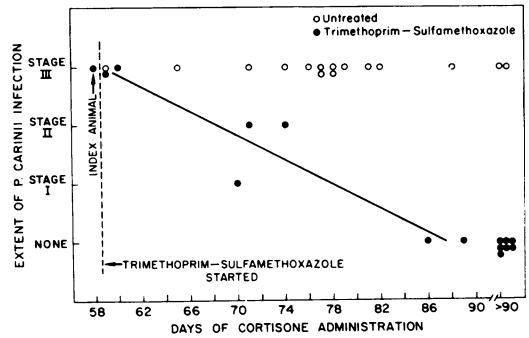


FIG. 1. Effect of trimethoprim-sulfamethoxazole in the treatment of established *P. carinii* infection. The index animal refers to the first animal of the group to die with *P. carinii* pneumonitis before treatment was started.

TABLE 1. Effects of trimethoprim-sulfamethoxazole in the prevention and treatment of *P. carinii* infection

Group	Mean wt (g)		Median day of survival	Extent of <i>P. carinii</i> infection ^a				Percent with <i>P. carinii</i>
	Initial	At death		None	Stage I	Stage II	Stage III	
Control (no cortisone) ^b	205	467	124	15	0	0	0	0%
Control (cortisone)	198	118	78	0	0	0	15	100%
Pentamidine	207	120	77	2	0	5	7 ^c	86%
Trimethoprim-sulfa therapy	206	135	88	9	1	2	2 ^c	36%
Trimethoprim-sulfa prophylaxis	203	115	96	15	0	0	0	0%

^a Staging of the extent of *P. carinii* infection is as follows. Stage I is characterized by isolated cyst forms in the cytoplasm of the cells of the alveolar septal wall or free in the alveolar lumen with absence of inflammatory or cellular response; stage II is characterized by an increase in the number of organisms with minimal septal inflammatory response and minimal response of alveolar macrophages; and stage III is characterized by extensive reactive and desquamative alveolitis with large numbers of cyst forms in the alveoli.

^b Sacrificed.

^c Index animal, stage III, untreated, not included.

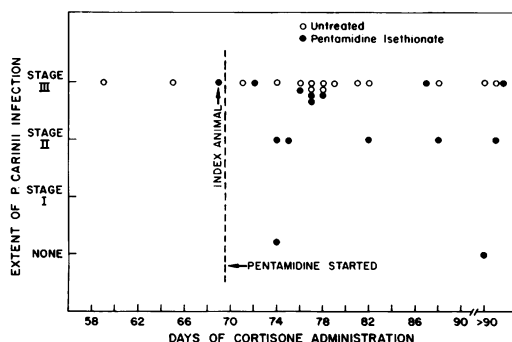


FIG. 2. Effect of pentamidine isethionate in the treatment of established *P. carinii* infection. The index animal refers to the first animal of the group to die with *P. carinii* pneumonitis before treatment with pentamidine was started.

pentamidine therapeutically, had survival times equal to or less than those of the untreated cortisone control animals. The incidence of *P. carinii* pneumonitis in these groups ranged from 80 to 100%.

The causes of deaths based on histological examination of lungs were determined. Group 1 (control, no cortisone) animals were sacrificed, and no significant evidence of infection was found. All of group 2 (cortisone controls, untreated) died with extensive stage III pneumonitis with little or no evidence of secondary infection. This group received tetracycline. Group 4 (trimethoprim-sulfamethoxazole, therapy) had three deaths from extensive *P. carinii* pneumonitis, three from mixed *P. carinii*, but predominantly bacterial, infection, and four from bacterial infection. Two of the five survivors had mild to moderately extensive bacterial pneumonitis at the time of sacrifice. The pentamidine-treated group 5 had eight deaths from *P. carinii* pneumonia, five from mixed bacterial and *P. carinii* infections, one from bacterial pneumonia, and one without infection. In group 3 (trimethoprim-sulfamethoxazole, prophylaxis), six died from bacterial pneumonia, one died from fungal (yeast) infection, two died from pneumonitis associated with viral inclusions, and in six the cause of death was not apparent.

The causes of deaths in the groups receiving clindamycin or rifampin, separately or with pentamidine, were similar to those of group 5 treated with pentamidine.

DISCUSSION

Under the conditions of these experiments, the trimethoprim-sulfamethoxazole combination is effective in the prevention and treatment of *P. carinii* pneumonitis in the rat. When

compared with pentamidine, trimethoprim-sulfamethoxazole is more effective therapeutically. Although the index animal in the pentamidine-treated group died 10 days later than the index animal in the trimethoprim-sulfamethoxazole-treated group, the animals in both groups were comparable as to weights and general appearance at the time treatments were begun. Furthermore, six of the pentamidine-treated animals died with *P. carinii* pneumonitis after receiving at least 10 days of treatment. Earlier administration or higher dosage of pentamidine might have improved the survival rates; however, the dosage used was the same as that shown to be effective in the treatment of *P. carinii* infection in humans (19) and rats (4) and in patients with trypanosomiasis. The extensive necrosis at injection sites encountered in our study, as well as others (4, 8), precludes the administration of pentamidine over prolonged periods of time. Frenkel et al. (4) found that the prophylactic use of pyrimethamine and sulfadiazine in rats had a more prolonged effect than pentamidine. Post et al. (13) were able to prevent *P. carinii* pneumonitis in infants under epidemic conditions by the administration of pyrimethamine and sulfadoxine. Pyrimethamine interferes with the conversion of folic to folinic acid.

In addition to therapeutic efficacy, trimethoprim-sulfamethoxazole offers the advantages of less toxicity than pentamidine, the potential for prolonged administration for prophylactic use, and oral route of administration in widely separated doses. Rats treated for 6 months with 100 mg of trimethoprim per kg per day and 400 mg of sulfamethoxazole per kg per day had no evidence of significant effects on leukocytes, platelets, and erythrocytes. However, a three-fold increase in dosage caused "slight to moderate falls" in erythrocyte and platelet counts (18).

Immunosuppressive effects of trimethoprim-sulfamethoxazole have been suggested by studies which have shown impairment of phytohemagglutinin-induced lymphocyte transformation (5), prolongation of skin allograft survival in mice (6), suppression of hypersensitivity to dinitrochlorobenzene (7), and suppression of antibody response in man (1). These parameters could not be investigated in the already immunosuppressed rats in our study.

In *P. carinii* pneumonitis, the organisms are found in abundance in the cytoplasm of alveolar macrophages (9). One study has suggested that trimethoprim-sulfamethoxazole has a stimulating effect on granulocyte metabolism (14). Sig-

nificant increases in measurable hydrogen peroxide production and oxygen uptake were obtained during phagocytosis of heat-killed *Staphylococcus aureus* by granulocytes of normal individuals as well as those from a patient with chronic granulomatous disease. This observation deserves further study, especially with the alveolar macrophage and *P. carinii*.

Pharmacokinetic studies of trimethoprim and sulfamethoxazole have demonstrated high concentrations of these drugs in the lungs of rats treated orally. Trimethoprim and sulfamethoxazole levels in lung tissue were two to three times those of plasma (17). Since with fatal *P. carinii* infection the organisms remained localized to the lungs, an antimicrobial agent with high perfusion capabilities of this organ is desirable.

Higher doses of trimethoprim and sulfamethoxazole than those used in this study would likely be tolerated without significant toxic effects and might perhaps be more effective in the treatment of *P. carinii* infection. Although trimethoprim and sulfamethoxazole were not studied separately, studies with other organisms (15) indicate that synergism occurs with a broad spectrum of organisms. Furthermore, pyrimethamine combined with sulfadiazine (4) was more effective with *P. carinii* infection than was either of these drugs alone.

In conclusion, our studies show that trimethoprim-sulfamethoxazole is an effective antimicrobial regimen for the prevention and treatment of murine *P. carinii* pneumonitis. Clinical studies with these drugs are warranted.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant AI-11277 from the National Institute of Allergy and Infectious Diseases, Childhood Cancer Research Center grant CA-08480, Public Health Service clinical cancer-medical training grant CA-08151 from the National Cancer Institute, and by American-Lebanese Syrian Associated Charities.

LITERATURE CITED

- Arvilommi, H., M. Vuori, and A. Salmi. 1972. Immunosuppression by co-trimoxazole. *Brit. Med. J.* **25**:761-762.
- Dauzier, G., T. Willis, and R. N. Barnett. 1956. *Pneumocystis carinii* in an infant. *Amer. J. Clin. Pathol.* **26**:787-793.
- Donno, L., and V. Sanguineti. 1970. Therapy of natural falciparum malaria with combinations of antifolic drugs in non-immune subjects. *Chemotherapy* **15**:118-124.
- Frenkel, J. K., J. T. Good, and J. A. Schultz. 1966. Latent pneumocystis infection in rats, relapse and chemotherapy. *Lab. Invest.* **15**:1559-1577.
- Gaylarde, P. M., and I. Sarkany. 1972. Suppression of thymidine uptake of human lymphocytes by co-trimoxazole. *Brit. Med. J.* **25**:144-146.
- Ghilchick, M. W., A. S. Morris, and D. S. Reeves. 1970. Immunosuppressive powers of the antibacterial agent trimethoprim. *Nature (London)* **227**:393-394.
- Helander, I., H. Arvilommi, S. Lund, and V. K. Hopsu-Havu, reference in Arvilommi, H. et al., 1972. Immunosuppression by co-trimoxazole. *Brit. J. Med.* **25**:761-762.
- Hughes, W. T., H. K. Kim, R. A. Price, and C. Miller. 1973. Attempts at prophylaxis for murine *P. carinii* pneumonitis. *Curr. Ther. Res.* **15**:581-587.
- Hughes, W. T., R. A. Price, H. K. Kim, T. Coburn, D. Grigsby, and S. Feldman. 1973. *Pneumocystis carinii* pneumonitis in children with malignancies. *J. Pediat.* **82**:404-415.
- Lewis, C. 1968. Antiplasmodial activity of halogenated lincomycin analogues in *Plasmodium berghei*-infected mice. *Antimicrob. Ag. Chemother.* **1967**, p. 537-542.
- Luneth, J. H., T. W. Kirmse, A. P. Prezyna, and R. Gerth. 1955. Interstitial plasma cell pneumonia. *J. Pediat.* **46**:137-145.
- McMaster, P. R. B., K. G. Powers, J. F. Finerty, and M. H. Lunde. 1973. The effect of two chlorinated lincomycin analogues against acute toxoplasmosis in mice. *Amer. J. Trop. Med. Hyg.* **22**:14-17.
- Post, C., T. Fakhouri, W. Dutz, B. Bandarizadeh, and E. E. Kohout. 1971. Prophylaxis of epidemic infantile pneumocystosis with a 20:1 sulfadoxine plus pyrimethamine combination. *Curr. Ther. Res.* **13**:273-279.
- Pyesmany, A. F., and D. L. Cameron. 1973. Septin-induced stimulation of granulocyte metabolism in chronic granulomatous disease. *Pediatr. Res.* **7**:371.
- Reisberg, B., J. Herzog, and L. Weinstein. 1967. In vitro antibacterial activity of trimethoprim alone and combined with sulfonamides. *Antimicrob. Ag. Chemother.* **1966**, p. 424-429.
- Remington, J. S., T. Yagura, and W. S. Robinson. 1970. The effect of rifampin on *Toxoplasma gondii*. *Proc. Soc. Exp. Biol. Med.* **135**:167-172.
- Schwartz, D. E., and J. Rieder. 1970. Pharmacokinetics of sulfamethoxazole plus trimethoprim in man and their distribution in the rat. *Chemotherapy* **15**:337-355.
- Udall, V. 1969. Toxicology of sulfaphonamide-trimethoprim combinations. *Postgrad. Med. J.* **45**(Suppl):42-45.
- Western, K. A., D. R. Perera, and M. G. Schultz. 1970. Pentamidine isethionate in the treatment of *P. carinii* pneumonia. *Ann. Intern. Med.* **73**:695-701.