

Combination of Pentamidine and Trimethoprim-Sulfamethoxazole in the Therapy of *Pneumocystis carinii* Pneumonia in Rats

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Treatment with either pentamidine isethionate or trimethoprim-sulfamethoxazole significantly reduces the mortality of *Pneumocystis carinii* pneumonia. It is not known whether a combination might act in an additive, synergistic, or antagonistic manner. We studied the interaction of these two agents in the steroid-conditioned rat model of pneumocystosis. Of animals receiving pentamidine alone, 48% died and 45% had *P. carinii* cysts at autopsy. Trimethoprim-sulfamethoxazole alone resulted in 21% mortality, and cysts were found in 28%. Both agents in full doses resulted in 45% deaths and cysts in 37%. Animals treated with half-dosages of pentamidine plus trimethoprim-sulfamethoxazole had mortality of 35%, and 21% had cysts. Trimethoprim alone, in two dosages, was ineffective in eradicating *P. carinii* cysts. The data suggest that combination therapy is no more effective than trimethoprim-sulfamethoxazole alone in the treatment of *P. carinii* pneumonia.

Pneumocystis carinii is a protozoan which causes opportunistic pulmonary infection in immunosuppressed patients. Until the introduction of pentamidine isethionate (7), mortality in immunocompromised hosts approached 100% (2, 12, 17). Pentamidine isethionate therapy has reduced mortality to 30 to 50% (8, 14, 15, 17). Because pentamidine is associated with a number of undesirable and toxic side effects, other therapeutic agents have been sought. Some success was noted using a combination of pyrimethamine and sulfadiazine (1, 9), but the results were not always consistent (11, 13). Experimental work by Hughes et al. (6) led to clinical trials of the fixed combination of trimethoprim-sulfamethoxazole in patients with *P. carinii* pneumonia (4, 10; W. T. Hughes, S. Feldman, and S. Chaudhary, *Pediatr. Res.* 10: 399A). These studies demonstrated survival rates equal to pentamidine and a lesser incidence of significant side effects.

The concomitant use of pentamidine isethionate and trimethoprim-sulfamethoxazole has not been studied. It is important to know whether such a combination has a therapeutic advantage without increased toxicity before it is administered to humans. The present study was designed to investigate the interaction of these two agents.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (Flow Laboratories, Rockville, Md.), weighing approximately 200 g were housed in a single room in the infectious disease area of the animal quarters. They were kept five to a cage and had free access to Purina Lab Chow and water. Cortisone acetate was used to provoke *P. carinii* pneumonia according to the method of Frenkel et al. (1). Doses of 25 mg were administered subcutaneously twice each week until spontaneous death or sacrifice of the animal (36 to 80 days). Tetracycline was administered in the drinking water in a concentration to result in each rat's consuming approximately 15 mg/day. This dose provided protection against infections with *Corynebacterium kutscheri*, reportedly a major cause of death in cortisone-conditioned rats (1).

Experimental design. Two separate studies were carried out, one with six treatment groups and one with eight treatment groups. There were no significant differences between the two experiments, so the data were combined for purposes of analysis. The animals were randomly assigned to groups of 15 for steroid conditioning. When the index animal in each group died and *P. carinii* pneumonia was histologically documented, therapy of the remaining animals in the group was initiated. The time elapsed between death and histological confirmation was always less than 10 h. The treatment groups are outlined in Table 1. Groups 2 through 8 received cortisone conditioning and tetracycline throughout the experimental period.

The various antimicrobial treatments were administered for 14 days, deaths were recorded, and all surviving animals were sacrificed at the end of this time. Surviving animals in group 2 were sacrificed 2 weeks after the index animal died of pneumocystis pneumonia. Six animals chosen at random from group 1 were sacrificed at the end of the experiment.

Antimicrobial agents. Pentamidine isethionate (obtained from the Center for Disease Control in Atlanta, Ga. and from Rhodia Inc., New York, N.Y.) was injected subcutaneously in a dosage of 20 mg/kg (4 mg per rat) or 10 mg/kg (2 mg per rat) each day.

Trimethoprim and sulfamethoxazole (kindly provided by Hoffman-LaRoche, Inc., Nutley, N.J.) were administered in the drinking water in such concentrations (based on predicted intake per rat per day) as would provide 50 mg of trimethoprim per kg (10 mg per rat) and 250 mg of sulfamethoxazole per kg (50 mg per rat) each day. Some animals received trimethoprim alone at concentrations of 50 mg/kg (10 mg per rat) or 10 mg/kg (4 mg per rat) daily. Measurement of blood levels was not done.

These antibiotic dosages are derived from the work of Frenkel et al. (1) and Hughes et al. (6) using similar experimental models of pneumocystis infection. The routes of administration are likewise considered standard in models of pneumocystis pneumonia in rats.

Pathology. The lungs were examined at the time of death; both impression smears and fixed sections were studied using toluidine blue O. Typical cysts were required for the diagnosis of *P. carinii* infection. The severity of the infection was assessed by two independent observers who did not know the identity of the individual slides. A system of 0 to 4 was used wherein 0 = no *P. carinii* cysts seen; 1/2 = rare cysts; 1 = a few cysts in a few alveoli; 2 = a few cysts in many alveoli; 3 = half the alveoli filled with a moderate number of cysts; 4 = more than half the lung involved (1). To further quantitate the extent of pneumocystosis a disease scoring index was developed: category 0 or 1/2 = 0, category 1 = 1, category 2 = 8, category 3 = 27, and category 4 = 64. The values for each

animal were added together and averaged to provide a group index.

Pentamidine toxicity. Twenty healthy male Sprague-Dawley rats weighing approximately 200 g each were marked and placed in cages in such a way that each rat could be identified. They were allowed free access to regular chow and fresh water. Animals were weighed on day 1 and then every day for the course of the experiment. Ten rats received daily subcutaneous injections of a 0.4% solution of pentamidine isethionate, 4 mg/day, and 10 rats received daily subcutaneous injections of sterile, normal saline in a volume equal to the pentamidine dose. No other drugs were administered. An observer who did not know which animals were receiving drug or placebo noted complications of therapy, on a daily basis, i.e., skin necrosis, abscess formation, or transient paralysis.

RESULTS

The experiment was started by administering cortisone acetate to groups of 15 animals. During the conditioning period, three rats died without determined cause and without evidence of pneumocystis at autopsy. Antimicrobial treatment was initiated only when an animal died with histologically documented pneumocystis pneumonia. In five other animals, autolysis of lung tissue prevented detection of *P. carinii* cysts. These animals were not included in the analysis, and they account for the discrepancy between beginning and analyzed group sizes.

The data are shown on Table 1. One of the negative control animals (group 1) died during the experiment, but neither this animal, nor six other negative controls sacrificed, had any evidence of *P. carinii* infection by histological examination of the lungs. There were only six deaths in the positive control group (group 2);

TABLE 1. *Fatalities, disease index, and extent of pneumocystosis in cortisone-conditioned rats receiving specific antimicrobial therapy with pentamidine isethionate and/or trimethoprim-sulfamethoxazole*

Group	Treatment schedule: daily dose (mg/kg) ^a			No. of rats in treatment group	Fatalities during treatment period		Survivor status at sacrifice		Total no. (%) with grade 1-4 ^c pneumocystosis
	PENT	TMP	SMZ		No. (%)	No. diseased (avg score) ^b	No.	No. diseased (avg score) ^b	
1				15	1 (6.6)	0 (0)	6	0 (0)	0 (0)
2-C ^d				28	6 (21.4)	5 (12.5)	22	16 (5.0)	21 (75.0)
3-C	4			29	14 (48.3)	9 (12.7)	15	4 (1.2)	13 (44.8)
4-C		10	50	29	6 (20.7)	5 (19.2)	23	3 (0.4)	8 (27.6)
5-C	4	10	50	29	13 (44.8)	10 (29.0)	14	0 (0)	10 (37.0)
6-C	2	10	50	29	10 (34.5)	5 (11.9)	19	1 (0.05)	6 (21.4)
7-C		10		14	5 (35.7)	5 (26.8)	9	8 (18.9)	13 (92.9)
8-C		4		14	5 (35.7)	4 (26.0)	9	6 (4.3)	10 (71.4)

^a PENT, Pentamidine isethionate; TMP, trimethoprim; SMZ, sulfamethoxazole.

^b No. diseased, Number of animals with pneumocystis demonstrated at autopsy; avg score, average disease scoring index (see the text).

^c Graded by number of cysts: 1, a few cysts in a few alveoli; 2, a few cysts in many alveoli; 3, half the alveoli filled with a moderate number of cysts; 4, more than half the lung involved.

^d C, Cortisone conditioned.

however, at the time of autopsy 21 (75%) had *P. carinii* pneumonitis.

Animals treated with pentamidine isethionate alone (group 3) suffered a 48% mortality, and at autopsy *P. carinii* cysts were still present in 45%. The number of rats with *P. carinii* cysts following pentamidine therapy was significantly lower than in the positive control group ($P = 0.02$ by χ^2). The average disease scoring index was also decreased among survivors. The pentamidine injections were associated with skin necrosis, apparent abscess formation, and paralysis of the extremity nearest the injection site in all animals.

Trimethoprim-sulfamethoxazole therapy given alone (group 4) resulted in the lowest mortality, 21%, and autopsy evidence of *P. carinii* pneumonitis was found in 28%. There was a significant decrease in the number of animals with pneumocystis in this group compared with the positive control group ($P < 0.001$ by χ^2). Among survivors, the average disease scoring index was much lower than that for either positive controls or pentamidine-treated animals, although not statistically significant.

Animals receiving trimethoprim-sulfamethoxazole plus pentamidine isethionate in full doses (group 5) had a mortality rate of 45% and *P. carinii* cysts on histological examination in 37%. When the half-dose of pentamidine isethionate was used in combination with trimethoprim-sulfamethoxazole (group 6), the mortality was 35% and *P. carinii* cysts were detected in 21%. Both combination therapy groups had significantly fewer rats with *P. carinii* cysts at autopsy compared with positive controls ($P < 0.01$ and $P < 0.001$, respectively, by χ^2). However, no significant differences were noted in comparing the combination groups with either agent alone. The average disease scoring index for each combination was lower than that of either drug alone.

Trimethoprim alone, administered at 10 mg (group 7) or 4 mg (group 8) daily, resulted in identical mortality rates (36%), and *P. carinii* cysts were demonstrated in 93% and 71%, respectively. The number of animals with pneumocystis was essentially the same as animals in the positive control group, and the average disease scoring index in survivors was the same or greater.

Healthy rats (non-steroid-conditioned) were used to detect adverse reactions to pentamidine. Daily injections of 4 mg of pentamidine isethionate (0.4% solution) uniformly resulted in bleeding, induration, and subsequent necrosis of skin. Several of the pentamidine group had transient paresis of the hind limb, which disappeared within 2 to 4 h. Of 10 rats receiving saline pla-

cebo, 8 did not develop signs of induration or necrosis. The remaining two rats inadvertently received a single injection of pentamidine; a small area of induration was detected over the injection site in these animals at the end of the experiment. During the study period, the treated animals averaged a smaller percent weight gain than the placebo group, but the difference was not statistically significant.

DISCUSSION

Six (21%) of the group 2 animals died during the experiment, a percentage considerably lower than that reported in the literature (5, 6, 16). This discrepancy is best explained by differences in the periods of steroid conditioning. In the present study, the positive controls were sacrificed when the last therapeutic group had received a 2-week course of treatment (54 and 80 days of cortisone conditioning), whereas other investigators have continued cortisone until all animals died (median 78 [6], 81 [16], and 93 [5] days of steroids). Since 21 of 28 animals in group 2 had histological evidence of *P. carinii* pneumonitis at autopsy, it is expected that continued steroids would have resulted eventually in the death of the animals from extensive pneumocystosis. In contrast, none of the group 1 animals had demonstrable *P. carinii* cysts.

Mortality rates were highest in groups 3 (48%) and 5 (45%), animals treated with pentamidine at 4 mg/day alone or in combination with trimethoprim-sulfamethoxazole. However, survivors in group 5 had a much lower disease scoring index than rats in group 3. This difference may relate to the inherent toxicity of pentamidine isethionate. Skin necrosis, abscess formation, and paralysis of the injected limb seen in our experimental animals have been noted by others (1, 5, 16). That pentamidine is the responsible agent was confirmed by the double blind study comparing pentamidine with a saline placebo in otherwise healthy animals. Even a single injection of pentamidine caused induration of the skin.

Animals treated with trimethoprim-sulfamethoxazole alone (group 4) had the lowest mortality, 21%, which was significantly lower than that for animals treated with pentamidine alone ($P = 0.05$ by χ^2). Statistically fewer animals in this group had *P. carinii* cysts demonstrated at autopsy compared with the untreated, cortisone-conditioned animals in group 2 ($P < 0.001$ by χ^2). The addition of pentamidine in half-dose to trimethoprim-sulfamethoxazole therapy (group 6) resulted in an increased mortality rate, although surviving animals did have a lower disease scoring index than those with either agent administered singly. Trimethoprim therapy

alone had little or no effect on mortality or in reducing the extent of pneumocystosis.

This study demonstrates that trimethoprim-sulfamethoxazole alone is the most effective therapy for *P. carinii* pneumonitis in rats as judged by survival and number of animals with cysts at autopsy. Extrapolation of animal model data to the human equivalent of disease must always be done with extreme caution, and it should be emphasized that the present study results are truly applicable only to rats. However, it appears that these data provide preliminary answers to some of the editorial questions posed by Hughes (3): namely, a combination of trimethoprim-sulfamethoxazole with pentamidine isethionate is no more effective in the treatment of *P. carinii* pneumonitis than trimethoprim-sulfamethoxazole alone, and may be, in fact, harmful; trimethoprim by itself has no place in the treatment of pneumocystosis.

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