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Monodrug Efficacies of Sulfonamides in Prophylaxis for *Pneumocystis carinii* Pneumonia

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A remarkably high rate of adverse events is associated with the use of trimethoprim-sulfamethoxazole in patients with human immunodeficiency virus type 1 infection. We examined the efficacies of sulfonamides alone in the prevention of *Pneumocystis carinii* pneumonitis, with the assumption that at least some of the adverse events with the drug combination might be due to trimethoprim. With the immunosuppressed rat model, eight sulfonamides were studied at 100, 10, and 1.0 mg/kg/day (10 rats per dosage and drug). *P. carinii* infection was prevented in all animals (100%) receiving dosages of as little as 1.0 mg of sulfamethoxazole, sulfamethoxypyridazine, and sulfadimethoxine per kg per day, as little as 10 mg of sulfameter, sulfachlorpyridazine, and sulfaquinoxaline per kg per day; and 100 mg of sulfaguanidine and sulfanilamide per kg per day. These studies suggest that a sulfonamide, such as sulfamethoxazole, might provide effective prophylaxis for *P. carinii* pneumonitis without trimethoprim.

Since the mid-1970s the combination of trimethoprim-sulfamethoxazole has been used extensively for the treatment and prevention of *Pneumocystis carinii* pneumonitis. When AIDS emerged in 1980, it was immediately obvious that *P. carinii* pneumonitis was a major component, affecting about 75% of patients. An unexpected event, still unexplained, was the remarkably high rate of adverse reactions to trimethoprim-sulfamethoxazole in AIDS patients. Reports describing adverse events in 44 to 69% of patients pointed to a serious limitation of the drug combination for individuals with human immunodeficiency virus type 1 infection (8, 14, 19, 20). The most frequent reactions included rash, neutropenia, and fever.

Two assumptions have gone unchallenged. First, because the adverse reactions to trimethoprim-sulfamethoxazole are similar to those known for half a century to occur with sulfonamides, it was assumed that sulfamethoxazole was the likely cause. Second, because trimethoprim plus sulfamethoxazole was shown to have synergistic antibacterial activity (5), the combination was expected to be synergistic for anti-*P. carinii* activity. Neither of these assumptions has been firmly confirmed or disproved with factual data. Some evidence suggests that at least some of the adverse reactions to trimethoprim-sulfamethoxazole might be due to the trimethoprim component (10, 11) and that sulfonamides alone have anti-*P. carinii* activity (7, 22).

The adverse effects associated with administration of trimethoprim alone are similar to those due to sulfonamides; these effects include rash, fever, neutropenia, and Stevens-Johnson syndrome. We have recently found adverse reactions to trimethoprim-sulfamethoxazole in AIDS patients to be more closely associated with serum trimethoprim concentrations than with serum sulfamethoxazole concentrations (10). A study by Leoung et al. (18) showed that adverse reactions were more frequent in AIDS patients receiving trimethoprim plus dapsone than in those receiving dapsone alone. Also, Carr et al. found that 16% of human immunodeficiency virus-infected individuals with prior adverse reactions to trimethoprim-sulfamethoxazole had recurrent reactions when challenged with trimethoprim alone (6). Both in vitro studies of *P. carinii* dihydrofolate reductase (2) and in vivo studies with an animal model (13) show that trimethoprim has little, if any, discernible anti-*P. carinii* activity. We reasoned that if at least some of the adverse reactions to trimethoprim-sulfamethoxazole are due to trimethoprim and if sulfamethoxazole alone is adequate for *P. carinii* prophylaxis, the single drug would have advantages over the drug combination in AIDS patients. If so, sulfonamides other than sulfamethoxazole should be investigated for anti-*P. carinii* activity to identify the compound with maximal potency and minimal toxicity.

The purpose of the study described here was to determine the relative efficacies of eight sulfonamides alone in the corticosteroid-immunosuppressed rat model for *P. carinii* pneumonitis. In many studies this animal model has been highly predictive of drug activity in humans with *P. carinii* infection (1, 9, 11, 16).

MATERIALS AND METHODS

Experimental design. The studies utilized immunosuppression of latently infected rats to provoke *P. carinii* pneumonitis. Sulfonamides were administered during the period of dexamethasone immunosuppressive therapy, and these rats were compared with untreated controls with respect to the extent of *P. carinii* pneumonitis seen at necropsy. Dosages of sulfonamides found effective in the prevention of *P. carinii* pneumonitis were decreased 10-fold and reevaluated. Similarly, drugs found effective at the reduced dose were again decreased 10-fold for additional evaluation. Deescalation of the dosage allowed the differentiation of the most potent from the least potent compounds.

Animals. Male, virus-free, Sprague-Dawley rats, weighing approximately 200 g, were obtained from area 202C at Harlan Sprague Dawley (Indianapolis, Ind.) and held in quarantine for 3 weeks. They were fed standard Purina rations and caged at five animals per cage.

Drugs. Dexamethasone sodium phosphate (Elkins-Sinn, Cherry Hill, N.J.) at 1.0 or 2.5 mg and tetracycline (Sumycin; Squibb, Princeton, N.J.) at 500 mg were added to each liter of drinking water. The medicated drinking water was made available continuously for ad lib access. Previous studies showed an average consumption of about 30 ml of drinking water daily. Dexamethasone was given throughout the 6-week study. The test drugs and dexamethasone were administered concomitently.

The following sulfonamides were purchased in powder form from Sigma Chemical Co. (St. Louis, Mo.): sulfamethoxazole, sulfadimethoxine, sulfamethoxypyridazine, sulfameter, sulfachlorpyridazine, sulfaquinoxaline, sulfaguanidine, and sulfanilamide (Fig. 1). For daily doses each drug was thoroughly mixed

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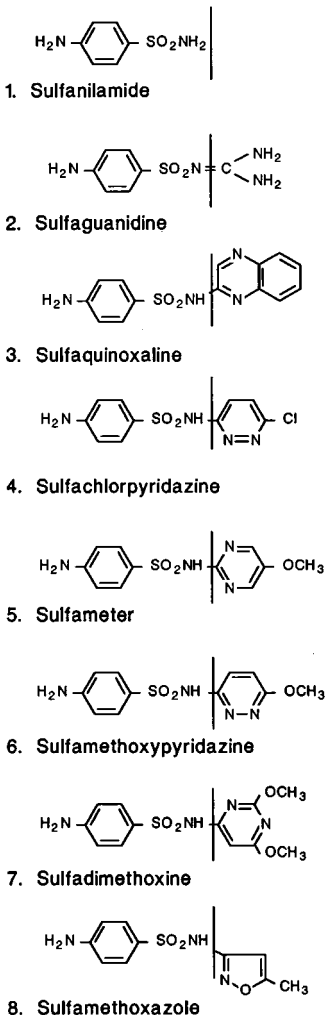

FIG. 1. Structural formulas for sulfonamides, showing variations upon the basic sulfanilamide.

into the daily ration of pulverized food and compounded into pellets. The drugs were evaluated at dosages of 100, 10, and 1 mg/kg/day.

Histology. At necropsy sections of the right lung were placed in 10% formalin and prepared in paraffin blocks for sections. Sections were stained by the Gomori-Grocott methenamine silver nitrate method. The coded slides were viewed for the presence or absence of *P. carinii*, and if *P. carinii* was present, the extent was graded from 1+ to 3+. A rating of 1+ indicated that cysts were found infrequently, with less than one organism per 25 high-power fields. The 2+ rating meant that focal areas of *P. carinii* pneumonitis were found surrounded by 10 to 25 high-power fields of normal lung. A 3+ rating was given to lungs diffusely infiltrated with *P. carinii* organisms in almost all fields observed. The slides were read by two examiners, and differences were resolved to the agreement of both investigators prior to decoding.

RESULTS

With dosages of 100.0 mg/kg/day, all of the sulfonamides were effective in the prevention of *P. carinii* infection, with the exception of sulfamethoxypyridazine, which prevented the infection in 80% of the animals (Table 1). In the two infected animals, the organism appeared atypical and degenerated. Five of the 10 untreated control animals had *P. carinii* pneumonitis of 3+ intensity.

When the dosage was reduced to 10.0 mg/kg/day, breakthrough *P. carinii* infection occurred in rats receiving sulfaguanidine and sulfanilamide. Early deaths occurred in 5 of the 10 animals given sulfaguanidine and in 8 of 10 given sulfanilamide prior to completion of the study, so these drugs were not tested further. In contrast to the results of the higher-dosage trial, none of the animals given sulfamethoxypyridazine had *P. carinii* infection.

The dosage of 1.0 mg/kg/day clearly delineated the superior efficacies of sulfamethoxazole, sulfadimethoxine, and sulfamethoxypyridazine. Each of these drugs was well tolerated when given over a 6-week period. Furthermore, in the two lower-dose experiments, animals were given 2.5 mg of dexamethasone per liter for more extensive immunosuppression, resulting in *P. carinii* pneumonitis of 3+ intensity in all of the untreated controls. Animals given the dosage of 100.0 mg/kg/day received only 1.0 mg of dexamethasone per liter.

DISCUSSION

The results of this study show that sulfonamides alone are highly effective in the prevention of *P. carinii* pneumonitis in the immunosuppressed rat model; that sulfamethoxazole, sulfamethoxypyridazine, and sulfadimethoxine have greater potencies than sulfameter, sulfachlorpyridazine, sulfaquinoxaline, sulfaguanidine, and sulfanilamide; and that dosages of the most potent drugs of as low as 1.0 mg/kg/day provide effective prophylaxis in 100% of animals studied.

The prime consideration is whether a sulfonamide alone might have a place in the prevention and treatment of *P. carinii* pneumonitis, and if so, which of the several sulfonamides is optimally suited for use in patients with human immunodeficiency virus type 1 infection. One can reason with some assurance that a sulfonamide alone would be no more effective than trimethoprim-sulfamethoxazole. However, it is also reasonable to expect that adverse reactions to a sulfonamide, such as sulfamethoxazole, alone might be less frequent than those to the drug combination of trimethoprim-sulfamthoxazole. Thus, the essential characteristic of a sulfonamide for monodrug usage in *P. carinii* pneumonitis would be an efficacy at least as great as that of trimethoprim-sulfamethoxazole but a significantly lower rate and magnitude of adverse reactions. The animal study described here provides strong evidence for the relative efficacies of eight sulfonamides but gives little insight into the possible toxicities that could be expected in humans.

The dosage of sulfamethoxazole currently used in the trimethoprim-sulfamethoxazole combination for *P. carinii* prophylaxis in humans is 25 mg/kg/day. Although one cannot assume a drug dosage to be equally effective in both humans and rats, the finding that only 1.0 mg of sulfamethoxazole per kg

TABLE 1. Comparison of anti-*P. carinii* activities of eight sulfonamides at three dosages

Treatment	No. with *P. carinii* pneumonitis at necropsy/no. studied (%) at the following dosage (mg/kg/day):		
	100	10	1.0
Sulfamethoxazole	0/10 (0)	0/9 (0)	0/10 (0)
Sulfadimethoxine	0/10 (0)	0/9 (0)	0/7 (0)
Sulfamethoxypryidazine	2/10[a] (20)	0/8 (0)	0/10 (0)
Sulfameter	0/10 (0)	0/10 (0)	6/10 (60)
Sulfachlorpyridazine	0/10 (0)	0/10 (0)	9/10 (90)
Sulfaquinoxaline	0/10 (0)	0/10 (0)	10/10 (100)
Sulfaguanidine	0/10 (0)	2/10 (20)	
Sulfanilamide	0/10 (0)	3/9 (33)	
None (control)	5/10 (50)	7/10 (70)	10/10 (100)

[a] Organisms appeared atypical and degenerated.

per day was totally effective in the prevention of *P. carinii* infection in rats suggests that a prophylactic monodrug regimen with a dosage no greater than that in current use would be adequate for prophylaxis. Whether a lower dosage would have equal efficacy in humans is not known. The usual therapeutic dosage for the sulfamethoxazole component of the combination is 100 mg/kg/day. Whether the dexamethasone and tetracycline given to provoke *P. carinii* pneumonitis in this study affected absorption, distribution, metabolism, and clearance is not known, but in this study all groups of rats were treated in the same manner.

In a study of several inhibitors of folic acid synthesis, Walzer et al. (22) found sulfadiazine at 250 mg/kg/day, sulfadoxine at 250 mg/kg/day, and sulfamethoxazole at 250 and 62.5 mg/kg/day to have anti-*P. carinii* activity in experimental rats. The fact that the addition of trimethoprim to sulfamethazole dosages of 250 or 62.5 mg/kg/day did not cause enhancement of activity over that of the sulfonamide alone is understandable in light of our study, in which only 1.0 mg/kg/day was highly effective.

Frenkel et al., in 1966, first reported the anti-*P. carinii* activity of a sulfonamide and its potentiation with pyrimethamine (7). They found that all (16 of 16) untreated rats developed *P. carinii* pneumonitis when immunosuppressed, while 88% (7 of 8) of those given pyrimethamine, 50% (4 of 8) of those given sulfadiazine, and 22% (2 of 9) of those given both sulfadiazine and pyrimethamine had the pneumonitis. The initial study of trimethoprim-sulfamethoxazole in 1974 (12) showed the combination to be effective in the prevention of murine *P. carinii* pneumonitis in all animals but did not evaluate the drugs separately. Kluge et al. (15) also found trimethoprim-sulfamethoxazole to be completely effective and trimethoprim alone to be ineffective, but the sulfonamide alone was not tested. Walzer et al. (22) also found trimethoprim alone to be ineffective in murine pneumocystosis.

The suggestion that trimethoprim might play a role in adverse reactions to the trimethoprim-sulfamethoxazole comes from a double-blind study to evaluate the therapeutic use of trimethoprim-sulfamethoxazole and atovaquone in 408 AIDS patients with *P. carinii* pneumonitis (10). Treatment-limiting adverse events occurred in 24% of those given trimethoprim-sulfamethoxazole. Furthermore, the incidence of anemia, neutropenia, and azotemia increased with increasing plasma trimethoprim concentration, while other adverse reactions, such as rash, fever, gastrointestinal symptoms, and liver dysfunction, were unrelated to the plasma drug concentration. No such relationship to the plasma sulfonamide concentration was discernible. The adverse effects attributed to trimethoprim from studies of both AIDS and non-AIDS populations include diarrhea, nausea, anorexia, vomiting, headache, leukopenia, anemia, methemoglobinemia, rash, pruritis, fever, malaise, and Stevens-Johnson syndrome (3, 4). The study by Carr et al. (6) of 31 human immunodeficiency virus-infected patients who had experienced adverse reactions to trimethoprim-sulfamethoxazole showed that 16% had adverse reactions when rechallenged with trimethoprim alone, while 58% of those given both trimethoprim and sulfamethoxazole in rechallenge had adverse reactions.

The sulfonamides selected for study included long-acting drugs that have been used fairly extensively in clinical practice. Only sulfamethoxazole is currently in general use, and it has replaced the once-popular drugs sulfadimethoxine and sulfamethoxypyridazime. The usual dosages are 60, 15, and 15 mg/kg/day, respectively. No clinical comparisons of the sulfonamide compounds studied here have been reported. Although a difference in the anti-*P. carinii* efficacies of the sulfonamides has been demonstrated, no evaluation of adverse effects was possible. It is likely that patients with hypersensitivity and idiosyncratic reactions to one of the sulfonamides will also have reactions to the others. However, differences in toxicity might occur. The sulfonamides are metabolized by several pathways. They are metabolized by N acetylation and oxidation to potentially toxic metabolites. Patients with severe adverse events tend to be slow acetylators (21). Evidence suggests that at least some of the adverse reactions to sulfonamides may be due to the interaction of metabolic pathways, possibly under genetic control, regulating N acetylation and specific detoxification of toxic metabolites of the drug.

Some differences between the three most effective drugs (sulfamethoxazole, sulfadimethoxine, and sulfamethoxypyridazine) and the other, less effective sulfonamides are notable. The absorptions of the sulfonamides studied are similar, with the exception of sulfaguanidine, which is poorly absorbed. Clearance of the drugs is most rapid with the short-acting sulfonamides, such as sulfanilamide, which was one of the least effective drugs against *P. carinii*. The three most effective drugs are medium-acting (sulfamethoxazole) or long-acting (sulfadimethoxine and sulfamethoxypyridazine) sulfonamides and are well absorbed, but clearance is slow. The degree of protein binding of sulfonamides varies and parallels the anti-*P. carinii* activity in this study. The short-acting sulfonamides, such as sulfadiazine and sulfanilamide, are approximately 20% protein bound; the medium-acting sulfamethoxazole is about 65% protein bound; and the long-acting sulfamethoxypyridazine and sulfadimethoxine are about 90% protein bound. The extent to which protein binding influences the rates of renal excretion of these drugs is not known (17). The percentage of each sulfonamide bound to serum protein is not constant, and when dissociation occurs, the drug is again available in an active form.

Because of the high predictive value of the animal model for human *P. carinii* pneumonitis and because sulfonamides have been used extensively as antibacterial agents, clinical trials to evaluate monodrug prophylaxis with a sulfonamide seem warranted. Although all of the sulfonamides studied here have not undergone comparative trials in humans, use of the earlier sulfonamides, such as the basic sulfanilamide, was associated with higher rates of adverse reactions than use of sulfamethoxazole. Thus, sulfamethoxazole would seem to be the most logical candidate for a monodrug trial.

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