ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 1986, p. 509–510
0066-4804/86/030509-02$02.00/0

Vol. 29, No. 3

Successful Treatment and Prevention of Murine *Pneumocystis carinii* Pneumonitis with 4,4'-Sulfonylbisformanilide

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Received 23 August 1985/Accepted 23 December 1985

***Pneumocystis carinii* pneumonitis was prevented in 0, 50, 100, and 100% of immunosuppressed rats given doses of 0.5, 5.0, 25.0, and 125.0 mg/kg (body weight) per day, respectively, of 4,4'-sulfonylbisformanilide (DFD). Therapeutic efficacy was demonstrated with DFD at 25.0 mg/kg per day, and when this dose was combined with trimethoprim, the combination was as effective as trimethoprim-sulfamethoxazole, which has been proven to be effective in the treatment of murine and human *P. carinii* pneumonitis.**

Recently, we found diaminodiphenylsulfone (dapsone) to be highly effective in the treatment and prevention of murine *Pneumocystis carinii* pneumonitis (6). Subsequently, two clinical studies in patients with acquired immunodeficiency syndrome showed dapsone in combination with trimethoprim (TMP) to be highly effective in the treatment of *P. carinii* pneumonitis (G. S. Leoung, J. Mills, W. Hughes, P. Hopewell, and C. Wofsy, Abstr. Int. Conf. Acquired Immunodeficiency Syndrome, abstr. no. W-25, 1985; S. Richardson, M. Fanning, J. Brunton, I. Salit, S. Read, and F. Shepherd, Abstr. Int. Conf. Acquired Immunodeficiency Syndrome, abstr. no. s7-4, 1985). Whereas some patients had adverse reactions to dapsone, others with and without intolerance to TMP-sulfamethoxazole (SMZ) were able to take dapsone uneventfully.

The purpose of this study was to evaluate the diformyl derivative of dapsone, 4,4'-sulfonylbisformanilide (DFD), for its anti-*P. carinii* effect. DFD is deformylated rapidly in the plasma (4) and liver (2) to diaminodiphenylsulfone. DFD has been found to exhibit a greater antimalarial activity in mice, on a weight basis, than dapsone (1) and to protect human volunteers challenged with *Plasmodium falciparum* (3). Also, tolerance studies in humans showed that escalating single doses up to 4,800 mg do not produce objective evidence of toxicity, suggesting a greater degree of safety than with dapsone.

When rats are immunosuppressed with dexamethasone for 6 weeks or longer and protected from bacterial infection by the administration of antibiotics, from 90 to 100% develop *P. carinii* pneumonitis that is easily demonstrated at autopsy. This animal model has been used previously for the study of dapsone and other antimicrobial agents in this infection (6). Drugs administered during the period of immunosuppression can be evaluated for anti-*P. carinii* activity by examination of the lung histologically at a point when a high proportion of the untreated controls are heavily infected with the organism. DFD was evaluated in this system by using the same experimental plan as described previously for the study of dapsone (6).

Four dose levels of DFD were studied. Doses of 0.5, 5.0, 25.0, and 125.0 mg/kg (body weight) per day were added to the daily food supply. DFD was obtained from the Jacobus Pharmaceutical Co., Inc., Princeton, N.J. It was tested in combination with TMP and compared with TMP-SMZ. TMP in a dose of 60 mg/kg per day (Proloprim; Burroughs Wellcome Co., Research Triangle Park, N.C.) was added to the daily food supply. TMP at 50 mg/kg per day and SMZ at 250 mg/kg per day were given in the drinking water (Bactrim suspension; Hoffmann-La Roche Inc., Nutley, N.J.).

The drug preparations were made freshly every other day for oral use. DFD was added to pulverized food pellets, following which a paste of the mixture was made, pressed into pellets, and allowed to dry. The weight of the food and volume of drinking water consumed daily were measured. The medicated water and food were available continuously until consumed. The amount of rations consumed during the previous 2 days was used to calculate the amount to prepare for the next 2 days. The usual food and water wastage was not measured but was estimated to be about 15% of the total. All of the medications were taken without difficulty.

Groups of 10 rats were given either 0.5, 5.0, 25.0, or 125.0 mg of DFD per kg per day during a 6-week period of immunosuppression with dexamethasone. A control group was immunosuppressed but did not receive DFD. The animals were sacrificed at the end of 6 weeks of study, and the lungs were examined for *P. carinii* pneumonitis. The results are given in Table 1. All of the controls were infected. The lowest dose of DFD, 0.5 mg/kg per day, had no discernible effect. The 5.0-mg dose prevented infection in half of the animals, establishing the effective dose for 50% of subjects. Doses of 25 and 125 mg/kg per day were totally effective.

Groups of 10 rats were given dexamethasone and tetracycline for 5 weeks, during which time *P. carinii* pneumonitis had become established. At this time, DFD was administered at dose levels of 0.5, 5.0, and 25.0 mg/kg per day, with and without TMP, for 3 weeks. The animals were sacrificed after 8 weeks of immunosuppression and 3 weeks of treatment. One group of animals served as untreated controls, and one group received TMP-SMZ, a drug known to be effective in the treatment of *P. carinii* pneumonitis. The results are summarized in Table 1. DFD was clearly effective at the dose level of 25.0 mg/kg per day in the treatment of animals with *P. carinii* pneumonitis when compared with the untreated control group. The combination of TMP with DFD was more efficient than DFD alone. The dosage of 25.0 mg/kg per day in combination with TMP was as effective therapeutically as TMP-SMZ.

Discussion. Previous studies showed almost identical results with dapsone at the same doses as used for DFD in this trial (6). Thus, whatever advantage DFD might have over

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TABLE 1. Evaluation of DFD for the prevention and treatment of *P. carinii* pneumonitis

Group[a], drug, and dose (mg/kg per day)	No. of rats studied[b]	No. of rats with the following extent of *P. carinii* pneumonitis at autopsy:		
		None	Focal	Extensive
Prevention				
Control, untreated	10	0	2	8
DFD, 0.5	10	0	3	7
DFD, 5.0	10	5	5	0
DFD, 25.0	10	10	0	0
DFD, 125.0	10	10	0	0
Treatment				
Control, untreated	10	0	0	10
DFD, 0.5	9	0	0	9
DFD, 0.5 + TMP, 60	6	0	1	5
DFD, 5.0	9	0	3	6
DFD, 5.0 + TMP, 60	9	1	1	7
DFD, 25.0	8	2	5	1
DFD, 25.0 + TMP, 60	9	7	1	1
TMP-SMZ	9	7	0	2

[a] All groups received dexamethasone and tetracycline daily throughout the experiment.

[b] Numbers indicate the animals surviving long enough to receive at least 2 days of DFD or TMP-SMZ treatment.

dapsone would likely stem from its lower toxicity and fewer side effects, if any. In a report by Heymann and Fieser (5), mention was made of Maier's findings that DFD is as effective as dapsone in the treatment of the avian form of malaria but DFD is less toxic.

Hemolysis and methemoglobin formation are two major side effects of dapsone administration which limit its utility. Kiese (7) and Weisburger and Weisburger (9) reviewed the mechanism of hemolysis and methemoglobin reduction. Sonntag et al. (8) reported preliminary data in dogs which support the suggestion that intravenous DFD produces less methemoglobin than does dapsone. They also reported that 90% of intravenous DFD is converted to dapsone in 9 h.

Human volunteer studies have provided information on the pharmacokinetics of DFD (8). After a single oral dose of 1,600 mg (estimated as 23 mg/kg for a 70-kg man), the maximum levels in plasma were 2.9 ± 0.6 μg/ml at 5 to 8 h with a half-life of 24 to 36 h. DFD is rapidly metabolized to yield dapsone. However, these workers also reported that DFD by mouth in malarial prophylaxis studies is poorly absorbed and suggested that DFD be given at least two to three times per week if levels in plasma greater than 0.5 μg/ml are to be maintained. No data are available for levels in the serum of rats given DFD orally. It cannot be assumed that levels in serum with the 23-mg/kg dosage mentioned above for man would be similar to those for the 25 mg/kg per day used in our rat study, because wide variations in drug levels may occur from one animal species to another.

DFD presents some problems in oral bioavailability, but it might be a better tolerated form than dapsone for parenteral administration. However, adequate human trials comparing DFD and dapsone have not been done.

This work was supported by Public Health Service grant AI 20673 from the National Institute of Allergy and Infectious Diseases.

Maurizio Strosselli provided technical assistance.

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