Efficacy of Diaminodiphenylsulfone and Other Drugs in Murine *Pneumocystis carinii* Pneumonitis

WALTER T. HUGHES* AND BESSIE L. SMITH

Division of Infectious Diseases, St. Jude Children's Research Hospital, Memphis, Tennessee 38101

Received 4 June 1984/Accepted 30 July 1984

The purpose of this study was to identify new drugs for the prevention and treatment of *Pneumocystis carinii* pneumonitis (PCP) induced in rats by continuous daily dosage with dexamethasone. Initially, test drugs were administered prophylactically as a screen for efficacy. Drugs were selected because of their known activity against certain protozoa and their tolerance in human usage. Doses were based on previous studies in rats or estimated from usage in humans and lower animals. Allopurinol (50 mg/kg per day), ketoconazole (25 mg/kg per day), difluoromethylornithine (2.5 g/kg per day), diloxanide (125 mg/kg per day, nifurtimox (100 mg/kg per day), suramin (20 mg/kg per day), melarsoprol (20 mg/kg per day), gentian violet (0.5 mg/kg per week, 5 and 50 mg/kg per day), primaquine (5.6 mg/kg per day) and chloroquine (37.5 mg/kg per day) were ineffective, whereas diaminodiphenylsulfone (daspone) (25 mg/kg per day) was totally effective in preventing the infection. Diaminodiphenylsulfone was then evaluated at dose levels of 5, 25, and 125 mg/kg per day and compared with trimethoprim-sulfamethoxazole (TMP-SMZ), given at 50 per 250 mg/kg per day orally. The two highest dose levels of diaminodiphenylsulfone and TMP-SMZ prevented the infection in all of the animals, and the lowest dose of diaminodiphenylsulfone prevented it in 40% of the rats. All of the untreated controls developed PCP. To determine therapeutic efficacy, animals with extensive PCP were treated for 2.5 weeks with diaminodiphenylsulfone or TMP-SMZ. Based on residual extensive pneumonitis at the completion of treatment, the pneumonitis was reduced to 50% by TMP-SMZ and to 25% by diaminodiphenylsulfone, whereas 100% of untreated controls had extensive PCP. When treatment was begun earlier in the course of the pneumonitis, diaminodiphenylsulfone was totally effective in eradicating the infection. These results suggest that diaminodiphenylsulfone is an effective drug for the treatment and prevention of murine PCP and that it is at least as effective as TMP-SMZ.

In 1974 we used an animal model to show that trimethoprim-sulfamethoxazole (TMP-SMZ) was effective in the treatment and prevention of *Pneumocystis carinii* pneumonitis (PCP) (14), and subsequent clinical trials in humans demonstrated excellent correlation of therapeutic efficacy and prophylactic capability with the animal experiments (12, 13). Recently, investigators from several medical centers have reported high rates of adverse reactions to TMP-SMZ in patients with the acquired immune deficiency syndrome (AIDS) treated for PCP (16; C. B. Small, C. A. Harris, R. S. Klein, and G. H. Friedland, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 23rd, Las Vegas, Nev., abstr. no. 630, 1983; J. Kovacs, J. Hiemenz, A. Macher, D. Stover, H. Murray, J. Shelhamer, C. Lane, A. Fauci, P. Pizzo, J. Parrillo, and H. Masur, 23rd ICAAC, abstr. no. 228, 1983; F. Gordin, G. Simon, C. Wofsy, and J. Mills, 23rd ICAAC, abstr. no. 631, 1983). Adverse reaction rates ranging from 44 to 83% have been encountered. The mechanism responsible for these untoward effects is unknown. The only alternative drug, pentamidine isethionate, is also associated with adverse effects in a high proportion of AIDS as well as non-AIDS patients.

Because of the current need for additional drugs for the treatment of this infection, we have applied the same experimental approach to identify other drugs with therapeutic effects against *P. carinii*. Because of the urgency for the treatment of AIDS patients, we have directed attention primarily to drugs that would be currently available for use

in humans. The results of studies executed in our laboratory are the subject of this report.

MATERIALS AND METHODS

Experimental plan. The experimental design was based on the premise that dexamethasone and tetracycline administered daily for 6 weeks or longer will almost invariably provoke PCP pneumonitis in rats provided that tetracycline is also used to prevent early deaths from bacterial infection (11). Drugs administered during the period of immunosuppression can be tested for efficacy, and results can be compared with results in untreated controls. Under these conditions, each drug was tested in a group of 10 animals. When a drug was found to be efficacious in preventing *P. carinii* infection, it was then retested in a similar manner at three dose levels and compared with TMP-SMZ. Thereafter, the drug of promise was evaluated for its effect in the treatment of pneumonitis, and results were compared with results in untreated controls and animals treated with TMP-SMZ, a drug of proven efficacy. Untreated control animals as well as those receiving the test drugs were maintained on dexamethasone and tetracycline throughout the experiments.

Animal model. Male Sprague-Dawley rats (Harlan Industries, Indianapolis, Ind.) weighing approximately 200 g and caged in groups of five were given standard rations and drinking water containing dexamethasone and tetracycline throughout the experiments. Those used for testing the effects of drugs in preventing the pneumonitis were sacrificed in a carbon dioxide chamber after 6 weeks of study. Those used for testing therapeutic efficacy were sacrificed

* Corresponding author.

after 8.5 weeks of study, although some died earlier. The animals were autopsied, and the lower lobe of the right lung was bisected. One portion was placed in Formalin and processed for two sections with the Grocott-Gomori methenamine silver nitrate stain. The cut section of the remaining portion was imprinted onto microscope slides which were air-dried and stained separately with toluidine blue O and a modified Wright polychrome stain (Camco Quick; American Scientific Products, McGaw, Ill.). The histological sections and imprints were coded and read by one of us (W. T. Hughes). If *P. carinii* was not found in the sections or on imprints, the infection was graded as "none." If sparse numbers of organisms were localized to small areas of lung parenchyma surrounded by several high-power fields of normal lung tissues, the infection was graded as "focal pneumonitis." The term "extensive" pneumonitis was used for lungs with *P. carinii* organisms in each high-power field and with extensive alveolitis. Usually, three drugs were tested in each experiment, and 10 rats served as untreated controls in each experiment.

Drugs. The drugs tested were selected because of their known activities against certain protozoa, since *P. carinii* is presumed to be a protozoan and since drugs known to be effective in treatment of the infection also have antiprotozoan activity. Knowledge of the cellular and molecular biology of *P. carinii* is too limited for a more rational approach.

(i) **Dexamethasone-tetracycline.** Dexamethasone sodium phosphate (Decadron; Merk Sharpe & Dohme, West Point, Pa.) (2.0 mg) and 500 mg of tetracycline hydrochloride (Sumycin; E. R. Squibb & Sons, Princeton, N.J.) were added to 1 liter of drinking water. Each rat consumed 30 to 50 ml of the medicated water each day.

(ii) **Allopurinol (4-hydroxypyrazolo[3,4-d]pyrimidine).** Each rat was given 50 mg of allopurinol (Zylorim; Burroughs Wellcome Co., Research Triangle Park, N.C.) per kg per day in 30 ml of drinking water. Allopurinol interferes with the growth of certain *Leishmania* spp. (18, 23) and *Trypanosoma* spp. (2). The dosage selected for use was estimated as a maximal tolerated dose based on product information from Burroughs Wellcome Co. describing a 13-week experiment in rats given 72.0 mg/kg per day orally, with the result that 2 of 10 animals died with renal tubular damage.

(iii) **Ketoconazole.** Ketoconazole (Nizoral; Janssen Pharmaceutica, Piscataway, N.J.) was administered daily at 25 mg/kg per day in 30 ml of drinking water. This drug and similar imidazoles have been shown to be effective in vitro against *Plasmodium* spp. (M. A. Pfaller, D. J. Krogstad, and J. Segal, 21st ICACC, Chicago, Ill., abstr. no. 273, 1981), *Leishmania* spp. (3), and *Trypanosoma* spp. (5). Chronic toxicity studies in rats have shown that daily doses of 20 to 40 mg of ketoconazole per kg per day for 3 to 12 months are tolerated with minimal toxicity (10).

(iv) **DL- α -Difluoromethylornithine.** DL- α -Difluoromethylornithine was given at 2.5 g/kg per day in 30 ml of drinking water. The drug was supplied by P. P. McCann, Merrell Research Center, Cincinnati, Ohio. This drug was evaluated because of its curative effects on trypanosomiasis in mice treated with doses up to a concentration of 2% of drinking water (1, 19). We selected a slightly lower dose because of the prolonged period of administration required in this study.

(v) **Diloxanide furoate (Furamide).** Diloxanide furoate was given at 125 mg/kg per day in 30 ml of drinking water. The drug was supplied by the Parasitic Disease Drug Branch, Centers for Disease Control, Atlanta, Ga. Furamide has been found effective at doses as low as 3 to 6 mg/kg per day

in weanling rats infected with *Entamoeba histolytica* (25). Since the dose of 125 mg/kg per day is nontoxic in rats, we elected to use this dose.

(vi) **Nifurtimox (Lampit, Bayer 2502).** Nifurtimox was administered as an oral suspension at 100 mg/kg per day. Nifurtimox was supplied by the Centers for Disease Control. It is used in the treatment of trypanosomiasis. Doses of 100 to 500 mg/kg per day for 5 to 10 days in mice are effective and nontoxic (informational material for physicians, Bayer 2502, Centers for Disease Control). The dose of 100 mg/kg per day was selected in our study because of the prolonged period of administration.

(vii) **Suramin (Bayer 205).** Suramin was administered intravenously at 20 mg/kg once weekly, beginning on the first day of the experiment. The drug was infused within 30 min after preparation of the solution. It was supplied by the Centers for Disease Control. Suramin has been used in the treatment of trypanosomiasis. The 50% lethal dose (LD₅₀) for mice is 620 mg/kg, and the 10% lethal dose (LD₁₀) is 420 to 500 mg/kg intravenously (9). The dose for rats was estimated empirically.

(viii) **Melarsoprol (Mel B).** Melarsoprol was given intravenously at 20 mg/kg once weekly, beginning on the first day of the experiment. The drug was supplied by the Centers for Disease Control. Melarsoprol has been used for the treatment of trypanosomiasis. For mice, the minimal curative dose is 10 mg/kg per day, and the LD₅₀ is 65 mg intraperitoneally (24). The dose for rats was estimated for this study.

(ix) **Gentian violet (crystal violet).** Two oral doses of 5 and 50 mg/kg per day and one intravenous dose of 0.5 mg/kg per week were administered, respectively, to three groups of rats. Crystal violet was obtained from Eastman Kodak Co., Rochester, N.Y., and was filtered (pore size, 0.22- μ m) for sterility. The volume of each parenteral dose was 0.5 ml, and the oral doses were added to the daily supply of drinking water. Gentian violet possesses trypanocidal activity (6). Intravenous doses of an 0.5% solution have been used in humans for the treatment of strongyloidiasis (21). The doses selected in this experiment for oral and intravenous use in rats was empirical.

(x) **Primaquine and chloroquine.** A combination in doses of 5.62 mg of primaquine and 37.5 mg of chloroquine per kg per day was administered orally. The fixed-drug combination (Aralen with primaquine, U.S.P.) was obtained from Winthrop Laboratories, New York, N.Y. This drug combination prevents infection with *Plasmodium falciparum* and effects suppressive cure of infections with *Plasmodium vivax*. The dosage used here, calculated on weekly intake, is approximately 60-fold the prescribed human dose.

(xi) **TMP.** TMP at 60 mg/kg per day (Proloprim; Burroughs Wellcome Co.) was added to the daily food supply.

(xii) **TMP-SMZ.** TMP at 50 mg/kg per day and SMZ at 250 mg/kg per day were administered in the drinking water. A suspension of the fixed drug combination (Bactrim; Hoffmann-LaRoche, Inc., Nutley, N.J.) was used. This drug was used as a control treatment in a dosage known to prevent PCP effectively (14).

(xiv) **4,4'-Diaminodiphenylsulfone (Dapsone).** Three dose levels of diaminodiphenylsulfone were studied. A low dose of 5 mg, a mid-dose of 25 mg, and a high-dose of 125 mg/kg per day were added to the daily food supplies. Diaminodiphenylsulfone was obtained from Jacobus Pharmaceutical Co. Inc., Princeton, N.J. Functioning as an antifol, it exhibits significant activity against infections with all *Plasmodium* species. Studies on subacute toxicity indicate that rats can tolerate oral doses of 150 mg/kg per day over a

period of 60 days (26). The initial dose used in the first phase of our study was 25 mg/kg per day.

Administration of drugs. Drug preparations for oral use were made freshly every other day. Water bottles were changed daily or on alternate days. Medicated food pellets were prepared by mixing drugs in pulverized food rations, wetting to a thick paste, and pressing into pellets, which remained solidly formed with drying. The volume of medicated water and the weight of food pellets consumed were measured daily. The animals were weighed biweekly. No adjustments in drug concentrations were made since as body weight decreased, the volume of water and quantity of food consumed decreased proportionately. The medicated water and food were continuously available until consumed. The amount of food consumed during the previous 2 days was used as the measure for the next 2 days. The amount of food and water wastage could not be estimated. All medications were readily taken as prepared. The rats were maintained on an automatically controlled cycle of light and dark. The amount of medications consumed per individual animal was calculated as an average of the five rats per cage. The intravenous preparations were administered by tail vein infusion over a period of 2 min.

RESULTS

The results will be described for the four phases of the study. In the first phase of the study, 10 test drugs administered throughout the 6-week period of immunosuppression were well tolerated, except for the intravenous and highest oral doses of gentian violet. The animals given these drugs died before PCP could be expected to occur and were not included in the analysis. Animals receiving the oral gentian violet preparation had severe diarrhea, and those given the parenteral drug had obvious deposits of dye in capillaries of the pulmonary parenchyma. The results of histological studies of the lungs revealed *P. carinii* infection in 10 of each recipients of allopurinol, ketoconazole, difluoromethylornithine, diloxanide, nifurtimox, suramin, melarsoprol, and primaquine-chloroquine, in 8 of 10 recipients of gentian violet, and in 0 of 10 recipients of diaminodiphenylsulfone. The results from the separate control groups were similar; of the total, 48 of 50 had PCP. The extent of the pneumonitis was similar for each of the test drug groups, except that treated with diaminodiphenylsulfone, and the drug groups were similar to the controls. In each drug group and control group, at least 7 of the 10 animals had PCP graded as extensive.

In the second phase of the study, diaminodiphenylsulfone was retested to confirm the previous results, to evaluate doses above and below the dose used in the first phase, and to make a comparison with TMP-SMZ. All drugs and doses were well tolerated (Table 1).

The third phase of the study determined the efficacy of diaminodiphenylsulfone and TMP-SMZ for the treatment of severe extensive PCP (Table 2). One animal in the TMP-SMZ group and two in each of the diaminodiphenylsulfone groups died with extensive PCP before the drugs were started, and one in the TMP-SMZ group and two in the high-dose diaminodiphenylsulfone groups died within 48 h after starting the drugs. These were excluded for the evaluation of therapeutic effects. Although residual *P. carinii* infection was found in all animals except one treated with diaminodiphenylsulfone, the extent of the pneumonitis was clearly less in the drug-treated groups than in the untreated group. All of the untreated animals had extensive pneumonitis, but only one-half of the TMP-SMZ-treated group and approximately

TABLE 1. Comparison of three dose levels of diaminodiphenylsulfone with TMP-SMZ administered prophylactically (phase 2)

Drug	Dose (mg/kg per day)	Rats with indicated degree of PCP/rats tested			Mean wt loss (g) ^a
		None	Focal	Extensive	
None (control) ^b	0	0/9	0/9	9/9	50
Diaminodiphenylsulfone	5	4/10	6/10	0/10	35
	25	10/10	0/10	0/10	37
	125	10/10	0/10	0/10	26
TMP-SMZ	50-250	10/10	0/10	0/10	39

^a Mean difference between prestudy and death weights.

^b One of 10 rats was cannibalized.

one-fourth of the diaminodiphenylsulfone-treated groups had infections of similar extent.

In the third phase experiment, it seemed likely that we initiated drug therapy too late in the course of the pneumonitis to demonstrate maximally a therapeutic effect on the pneumonitis and eradication of the infection. Also, it seemed reasonable to expect that a pyrimidine, such as TMP, might have synergistic activity with the sulfone, especially if diaminodiphenylsulfone acts on microbial folate synthesis of *P. carinii*.

A fourth experiment was then designed to treat the pneumonitis in earlier stages. To decrease the intensity of the infection, the dose of dexamethasone was reduced to approximately one-half the dose used in the other experiments, and treatment was begun after 5 weeks of immunosuppression, rather than 6 weeks, in the preceding study. Also, diaminodiphenylsulfone was tested at three dose levels alone and in combination with TMP. One group received TMP alone, and one control group was not treated. The animals were sacrificed after 3 weeks of treatment rather than after 2.5 weeks as in the previous experiment. The results are summarized in Table 3. In all, 9 of the 10 controls and 8 of the 10 TMP-treated animals had PCP at autopsy. With the low dose of 5 mg of diaminodiphenylsulfone per kg per day alone, only 5 of 10 animals remained infected. However, when these two drugs were combined in the same dosages, only 1 of the 10 had detectable infection, suggesting some degree of synergy. The two higher doses were totally

TABLE 2. Residual PCP after 2.5 weeks of treatment with diaminodiphenylsulfone or TMP-SMZ compared with no treatment (phase 3)

Drug	Dose (mg/kg per day)	Rats dead within 48 h ^a	Rats with indicated degree of PCP/rats tested		
			None	Focal	Extensive
Control	0	1 ^b	0/9	0/9	9/9
Diaminodiphenylsulfone— ^c	25	2	0/8	6/8	2/8
	125	4	1/5	3/5	1/5
TMP-SMZ	50-250	2	0/8	4/8	4/8

^a Died within 48 h after first dose of drug.

^b Died after 6 weeks of immunosuppression because of bacterial infection; had focal PCP.

^c One of 10 diaminodiphenylsulfone-treated rats was cannibalized.

TABLE 3. Evaluation of diaminodiphenylsulfone in early treatment of PCP with and without TMP

Treatment ^a	Rats	
	No. studied	No. with PCP (after 3-wk course)
Untreated control	10	9
TMP (60 mg)	10	8
DDS (5 mg)	10	5
TMP + DDS (5 mg)	10	1
DDS (25 mg)	10	0
TMP + DDS (25 mg)	10	0
DDS (100 mg)	10 ^b	0
TMP + DDS (100 mg)	10 ^b	0

^a Numbers in parentheses show dose of drug in milligrams per kilogram per day. DDS, Diaminodiphenylsulfone.

^b One animal was cannibalized.

effective in eradicating the infection, either given alone or in combination with TMP.

DISCUSSION

The susceptibility of *P. carinii* to diaminodiphenylsulfone has been demonstrated in that doses of 25 and 125 mg/kg were totally effective in preventing the infection which occurred in all of the untreated control animals. Since the dose of 5 mg/kg per day prevented the infection in 40% of animals, the minimal effective dose required to prevent the infection in 100% of animals is between 5 and 25 mg/kg. Doses of 25 and 125 mg/kg per day were equally effective in the treatment of extensive pneumonitis. Thus, it appears that diaminodiphenylsulfone at doses of 25 to 125 mg/kg per day is as effective in the prevention and treatment of murine PCP as TMP-SMZ at a dose of 50 to 250 mg/kg per day. Only one dose level of TMP-SMZ was tested since this dose was shown to be effective in a previous study (14). These results are similar to our earlier animal study with TMP-SMZ. This latter study proved to be highly predictive of therapeutic and prophylactic responses in humans (12, 13). It is therefore reasonable to expect that animal studies of diaminodiphenylsulfone might be equally predictive for *P. carinii* infection in humans.

The doses of diaminodiphenylsulfone used in rats may not necessarily be applicable to humans. The doses used here are not excessive for rats. The oral LD₅₀ for rats is about 630 mg/kg (26). However, the plasma half-life in rats has been reported to be about 6 h (20), whereas ranges between 14 and 53 h have been found in humans (22). A recommended dosage for the treatment of leprosy in humans is 6 to 10 mg/kg per wk, which may average about 1.0 mg/kg per day.

Diaminodiphenylsulfone is now used almost exclusively for the treatment of dermatitis herpetiformis and leprosy. We selected it for trial with *P. carinii* because of its similarity to sulfonamides. The mechanism of action has not been established for *P. carinii*, but it is probably similar to that of the sulfonamides and probably affects folate synthesis.

The frequency and type of adverse reactions are of major concern in considering a new drug for use against this disease. Although the usual adult doses of 100 mg of diaminodiphenylsulfone daily in normal individuals and 50 mg daily in those with glucose-6-phosphate dehydrogenase deficiency do not cause hemolysis, larger doses are frequently associated with this abnormality (4). Methemoglobinemia is not uncommon. Less common adverse reactions include leukopenia, agranulocytosis, dermatitis, an infectious mononucleosis-like syndrome, fever, nausea, vomiting, abnormal-

ities in liver function tests, peripheral neuropathy, and renal papular necrosis (8). With appropriate monitoring, the drug has been used with relative safety in the treatment of leprosy, for which courses of at least two years and longer are required. Because of the peculiarities of drug tolerance in AIDS patients, the extent of adverse reactions cannot be predicted. It is hoped that it will provide a safe alternative for some or all of those who cannot take TMP-SMZ. An additional approach warranting further investigation is the utilization of a pyrimidine in combination with diaminodiphenylsulfone with the aim for therapeutic synergism and reduction in the dose of diaminodiphenylsulfone. Our study suggests that TMP offers promise in this regard.

Since many of the adverse effects of diaminodiphenylsulfone are dose dependent, the possible combination with TMP might permit further reduction in dosage. Compared with studies of sulfonamides alone (7, 17), diaminodiphenylsulfone alone would appear to be more effective. Also, derivatives of this parent compound having less toxicity should be tested.

Of the other drugs tested in this study, none showed promise of efficacy for the treatment of *P. carinii* infections. However, these were tested at only one dose level, which may or may not represent the optimal dose in rats. Evaluation of gentian violet was limited because of toxicity by the parenteral route and the possibility of nonabsorption of the tolerable oral dosage.

Based on the results of this study, previous correlations with therapy in the *P. carinii* animal model and responses in humans and adequate experience with the fairly safe use of the drug in humans, it seems reasonable to suggest that clinical trials to evaluate diaminodiphenylsulfone for the treatment and prevention of PCP in humans are warranted. Such trials must be carefully designed and controlled to assure objective evaluation of both therapeutic responses and adverse reactions. Pharmacokinetic studies in the immunocompromised host are needed. Enrollment of therapeutic failures to TMP-SMZ and pentamidine may not provide a fair appraisal of the drug since one of our animal experiments was perhaps handicapped by starting diaminodiphenylsulfone therapy late in the course of the pneumonitis. Studies in patients who are at very high risk for *P. carinii* or have early mild cases of the pneumonitis and who are known to react adversely to the established drugs might be justified. Indiscriminate use of diaminodiphenylsulfone in humans should be avoided until adequate studies are done.

Since severely immunocompromised hosts with AIDS and PCP frequently suffer from concomitant infections, additional benefits from the antibacterial effects of diaminodiphenylsulfone, including those against *Mycobacterium* spp., may warrant study. Furthermore, the derivative 4-amino-4'-hydroxylaminodiphenylsulfone has been employed as an oxidant to improve the metabolic and bactericidal functions of polymorphonuclear neutrophils from patients with chronic granulomatous disease (15). Whether or not this phenomenon plays a role in the host response in diaminodiphenylsulfone-treated PCP is not known.

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