ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 1992, p. 1943–1950
0066-4804/92/091943-08$02.00/0

Vol. 36, No. 9

Treatment of Experimental Pneumocystosis: Review of 7 Years of Experience and Development of a New System for Classifying Antimicrobial Drugs

PETER D. WALZER,[1,2*] JILANNA FOY,[2] PAUL STEELE,[3] AND MICHAEL WHITE[4]

Cincinnati Veterans Affairs Medical Center,[1] Division of Infectious Diseases, Department of Internal Medicine,[2] Department of Pathology and Laboratory Medicine,[3] and Division of Biostatistics, Department of Environmental Health,[4] University of Cincinnati College of Medicine, Cincinnati, Ohio 45267*

Received 18 March 1992/Accepted 6 July 1992

Over a 7-year period, we analyzed 261 dose regimens of antimicrobial drugs in the treatment and prevention of *Pneumocystis carinii* pneumonia in an immunosuppressed rat model. These compounds ranged from drugs in clinical use to newly synthesized agents. Drug efficacy was expressed as the magnitude of the reduction in median *P. carinii* cyst or nucleus counts on a scale ranging from inactive (<5-fold) to very markedly active (≥1,000-fold). The classification system was reproducible and allowed drugs studied at different times to be compared with each other. The system demonstrated a hierarchy of anti-*P. carinii* activity not only among classes of compounds but also among individual members of a drug class. Sulfonamides, sulfones, and diamidines were the most active agents; some purine nucleosides and nitrofurans also showed promising activity; and most antiparasitic, antifungal, antibacterial, and antiviral drugs were inactive. We conclude that this classification system represents a simple, quantitative method of comparing the activities of antimicrobial drugs against *P. carinii*. Information gained from this system should be helpful in developing new anti-*P. carinii* compounds and establishing standard procedures for their evaluation.

Over the past decade, there has been a major rise in the number of cases of *Pneumocystis carinii* pneumonia associated with AIDS. The well-publicized limitations of currently available anti-*P. carinii* drugs in AIDS patients have emphasized the need for new therapeutic approaches (37). Most drug testing has been performed in an immunosuppressed rat model of pneumocystosis, which accurately predicts activity against human *P. carinii*. Compounds which have exhibited anti-*P. carinii* activity in the rat model include antifolate drugs (alone or in combination) such as dihydrofolate reductase (DHFR) inhibitors, sulfonamides, sulfones, and sulfonylureas (7, 9, 12, 14, 16–20, 23, 24, 27, 35, 39, 42, 43); sulfonamides in combination with other drugs (e.g., macrolides) (13); diamidines and related cationic compounds (8, 11, 21, 34, 40); 8-aminoquinolones, alone or in combination with other agents (1, 28); purine nucleosides (1a, 32, 36); polyamine inhibitors (3, 4); nitrofurans (38); β-glucan inhibitors (26, 29, 30); hydroxynaphthoquinones (14); fluoroquinolones (2); iron chelators (4); and immunological agents (antibodies, cytokines) (10, 31).

Although the rat model has been very valuable, evaluation of anti-*P. carinii* drugs is time-consuming, expensive, and labor intensive; only a few compounds can be tested in a given experiment. Treatment studies have been performed by different experimental protocols and methods of evaluating drug efficacy; this lack of standardization has prevented the results of one investigator from being compared directly with those of another. Little attention has also been devoted to quantitating the reduction in organism burden which can be achieved with different doses of anti-*P. carinii* drugs. In addition, there have been few published sources of information of the compounds which have been investigated but have not shown activity against *P. carinii* (12).

* Corresponding author.

We have had a long-term interest in experimental pneumocystosis and, since 1984, have had a National Institutes of Health contract to test anti-*P. carinii* drugs in the rat model. In the present study, we review this experience and describe a new system for classifying the activity of anti-*P. carinii* drugs.

MATERIALS AND METHODS

Drugs. Some compounds were purchased commercially, whereas other were obtained from individual investigators, from pharmaceutical firms, or through the Developmental Therapeutics Branch, Division of AIDS, National Institute of Allergy and Infectious Diseases.

Animal protocol. The animal model used in these studies has been described in detail previously (22, 36, 37, 41). Most of the experiments involved treatment of pneumocystosis, although a few prophylaxis studies were also performed.

Evaluation of drug efficacy. Evaluations of drug efficacy, which were described in our earlier studies (5, 6, 22, 36, 38–40), were based on analysis of the severity of pneumocystosis in the lungs by quantitation of *P. carinii* cysts and nuclei and by a histologic scoring system.

Classification system. Over the 7-year period, 37 experiments were conducted, including 34 treatment studies, 2 prophylaxis experiments, and 1 combined treatment and prophylaxis study. The data base was composed of the following: (i) 261 drug dose groups consisting of 3,966 rats (these groups included drugs tested individually or in combination at one or more doses), (ii) 37 control steroid (C/S) groups consisting of 701 animals, and (iii) 36 control normal groups composed of 231 rats which ate a normal diet, drank plain tap water, and received no medications.

One goal of the classification system was to develop a method of expressing anti-*P. carinii* activity of drugs in a quantitative manner, i.e., on the basis of the magnitude of

TABLE 1. Drugs evaluated in the treatment of *P. carinii* pneumonia

Drug	Dose regimen (mg/kg/day)[a,b]
Antibiotics	
Ampicillin	150 (i.m.)
Chloramphenicol	20 (i.m.)–400 (p.o.)
Ciprofloxacin	100 (p.o.)
Erythromycin	25 (i.m.)–400 (p.o.)
Gentamicin	10 (i.m.)
Imipenem	100 (i.m.)
Rifabutin	100–200 (p.o.)
Spectinomycin	100 (i.m.)
Antifungal agents	
Amphotericin B	6 (s.c.)
Flucytosine	150 (p.o.)
Griseofulvin	100–300 (p.o.)
Miconazole	100 (s.c.)
Antiviral agents	
Acyclovir	100 (i.m.)
Amantadine	50–100 (p.o.)
AZT	15–100 (p.o.)
Dideoxyinosine	500 (s.c.)
Isoprinosine	250–500 (p.o.)
Purine nucleosides	
9-Deazainosine	7.5 (s.c.)–50 (i.p. 5 days/wk)
Purine nucleoside 1	15 (s.c.)–50 (i.p.)
Purine nucleoside 2	25 (i.p.)
Purine nucleoside 3	25 (i.p.)
Purine nucleoside 4	25 (i.p.)
Purine nucleoside 5	20 (i.p.)
Sulfonamides/sulfones	
Dapsone	5–125 (p.o.)
Sulfadiazine	250–500 (p.o.)
Sulfadoxine	250 (p.o.)
SMX	0.3–500 (p.o.)
DHFR inhibitors	
Diaveridine	100 (p.o.)
Pyrimethamine	3–18.75 (p.o.)
TMP	3–100 (p.o.)
DHFR inhibitor 2	10–30 (p.o.)
DHFR inhibitor 3	10–100 (p.o.)
DHFR inhibitor 4	10–30 (s.c.)
Nitrofurans/ nitroimidazoles	
Benznidazole	20 (p.o.)
Furazolidone	50–200 (p.o.)
Metronidazole	100 (s.c.)–600 (p.o.)
Nifurtimox	50–400 (p.o.)
Nitrofurantoin	100–200 (p.o.)
Nitromidazole 1	12.5–100 (i.m.)
Nitromidazole 2	12.5–100 (i.m.)
Diamidines	
Amicarbalide	1.5 (s.c.)–10 (s.c.) 3–7 days/wk)
Dimethylstilbamidine (*cis*)	4 (s.c.) 3 days/wk)
Dimethylstilbarmidine (*trans*)	4 (s.c. 3 days/wk)
Diminazine	2.5–20 (s.c.)
Ethidium bromide	0.5 (i.p.)–3 (i.m. 3–7 days/wk)
Guanylhydrazone	25 (s.c.)–75 (i.m. 3–7 days/wk)
Guanylhydrazone A1	5 (s.c.)–10 (s.c. 3–5 days/wk)
Guanylhydrazone A2	2.5 (s.c.)–10 (s.c. 3–5 days/wk)
Guanylhydrazone A3	3 (s.c.)–10 (s.c. 3–5 days/wk)
Guanylhydrazone B1	0.5 (s.c.)–30 (p.o.)
Guanylhydrazone B2	0.5 (s.c.)–30 (p.o.)
Guanylhydrazone B3	3 (s.c.)
Guanylhydrazone B4	0.5–3 (s.c.)
Guanylhydrazone B5	0.5–3 (s.c.)
Imidocarb	2.5–25 (s.c.)
Isometamidium	0.5 (i.m.)–3 (i.m. 3–7 days/wk)
Pentamidine	10 (i.m.)–20 (i.m. 3 days/wk)
Propamidine	5 (i.m.)–10 (i.m. 3 days/wk)
Arsenicals/antimonials	
Arsenical 1	1–2 (s.c.)
Arsenical 2	2 (s.c.)
Arsenical 3	1–2 (s.c.)
Arsenical 4	5 (s.c.)
Arsenical 5	2.5–5 (s.c.)
Arsenical 6	15 (s.c. 5 days/wk)
Arsenical 7	10 (s.c. 5 days/wk)
Astiban	10 (i.m. 2 days/wk)
Melarsoprol	10 (i.p. 3 days/wk)
Pentostam	10 (i.m.)
Other antiparasitic drugs	
Amprolium	50 (p.o.)
Bithionol	50 (p.o.)
Chlorpromazine	10–20 (p.o.)
Dehydroemetine	1–5 (i.m.)
Diethylcarbamazine	100 (p.o.)
DFMO	2–4% solution in drinking water
Furamide	300 (p.o.)
MDL 27695	3 (i.m.)–15 (s.c. 3–7 days/wk)
Monensin	5–10 (p.o.)
Praziquantel	100 (p.o.)
Quinacrine	6 (p.o.)
Quinidine	25 (p.o.)
Quinine	25 (p.o.)
Combinations	
Amphotericin B + flucytosine	12 (s.c.) + 300 (p.o.)
Amphotericin B + miconazole	6 (s.c.) + 100 (s.c.)
DFMO + diminazine	2–4% solution + 2.5–5 (s.c.)
DFMO + pentamidine	2–4% solution + 10 (i.m. 3 days/wk)
DFMO + MDL 27695	1–2% solution + 3–7.5 (i.m.)
Flucytosine + miconazole	150 (p.o.) + 100 (s.c.)
Dapsone + TMP	15 (p.o.) + 50 (p.o.)
Dapsone + diaveridine	15 (p.o.) + 50 (p.o.)
Dapsone + pyrimethamine	125–250 (p.o.) + 3–9 (p.o.)
Quinine + clindamycin	25 (p.o.) + 400 (p.o.)
Sulfadiazine + diaveridine	250–500 (p.o.) + 50–100 (p.o.)
Sulfadiazine + pyrimethamine	250 (p.o.) + 3–9 (p.o.)
Sulfadiazine + TMP	250–500 (p.o.) + 50–100 (p.o.)
Sulfadoxine + diaveridine	250 (p.o.) + 50 (p.o.)
Sulfadoxine + pyrimethamine	250 (p.o.) + 3–9 (p.o.)
Sulfadoxine + TMP	250 (p.o.) + 50 (p.o.)
SMX + diaveridine	250–500 (p.o.) + 50–100 (p.o.)
SMX + pyrimethamine	62.5–500 (p.o.) + 3–18.75 (p.o.)
SMX + TMP	3–500 (p.o.) + 0.6–100 (p.o.)
SMX + TMP + AZT	62.5 (p.o.) + 12.5 (p.o.) + 15–100 (p.o.)
SMX + DHFR inhibitor 1	500 (p.o.) + 100 (i.m.)

[a] Rats receiving regimens containing pyrimethamine were also given folinic acid (7 to 15 mg/kg s.c.).

[b] Abbreviations: i.m., intramuscularly; p.o., orally; s.c., subcutaneously; i.p., intraperitoneally.

reduction in organism burden. The other goal was to be able to compare the activities of drugs investigated in different studies performed at different times. On the basis of the consistent level of infection and results with compounds such as trimethoprim-sulfamethoxazole (TMP-SMX) in the animal model, we reasoned that both goals could be met by

TABLE 2. Classification of drugs in the treatment of *P. carinii* pneumonia by cyst counts

Drug classification	Ratio[a]
Very marked activity	
Dapsone	638[b]
Sulfadiazine	1,200
Sulfadiazine + diaveridine	3,600
Sulfadiazine + TMP	5,300
Sulfadoxine + diaveridine	2,600
Sulfadoxine + pyrimethamine	4,800
SMX	6,300
SMX + AZT	2,400
SMX + diaveridine	7,890
SMX + pyrimethamine	7,890
SMX + TMP	7,890
SMX + TMP + AZT	1,700
SMX + DHFR inhibitor 1	7,900
Guanylhydrazone B1	1,280
Guanylhydrazone B4	1,280
Marked activity	
9-Deazainosine	197
Diminazine	143
Guanylhydrazone A2	125
Imidocarb	450
Quinapyramine	806
Sulfadiazine + pyrimethamine	538
Sulfadoxine	789
Sulfadoxine + TMP	278
Moderate activity	
Amicarbalide	94
Dapsone + pyrimethamine	60
Dehydroemetine	14
Furazolidone	45
Isometamidium	30
Pentamidine	96
Pentamidine + DFMO	10
Propamidine	26
Guanylhydrazone B2	91
Guanylhydrazone B5	91
Slight activity	
Amantadine	5
Dimethylstilbamidine (*cis*)	6
Diminazine + DFMO	6
Metronidazole	5
Nitrofurantoin	5
Purine nucleoside 1	9
Purine nucleoside 2	7
No activity	
Acyclovir	3
Amphotericin B	<1
Amphotericin B + flucytosine	<1
Amphotericin B + miconazole	<1
Ampicillin	<1
Amprolium	1
Astiban	2
AZT	3
Benznidazole	1
Bithionol	1
Chloramphenicol	<1
Chlorpromazine	<1
Ciprofloxacin	<1
Dideoxyinosine	<1
DFMO	3
DFMO + MDL 27695	3
Dapsone + draveridine	1
Dapsone + TMP	2
Diaveridine	3
Diethylcarbamazine	1
Dimethylstilbamidine (*trans*)	1
Erythromycin	2
Ethidium bromide	1
Flucytosine	<1
Furamide	<1
Gentamicin	2
Griseofulvin	2
Guanylhydrazone	3
Guanylhydrazone A1	2
Guanylhydrazone A3	3
Imipenem	<1
Isoprinosine	1
Melarsoprol	<1
Miconazole	1
Miconazole + flucytosine	<1
Monensin	<1
Nifurtimox	3
Ornidazole	<1
Pentostam	<1
Praziquantel	3
Pyrimethamine	4
Quinacrine	1
Quinidine	<1
Quininine	<1
Quinine + clindamycin	3
Rifabutin	1
Spectinomycin	1
Spiramycin	1
Thiabendazole	<1
TMP	<1
Arsenical 1	<1
Arsenical 2	<1
Arsenical 3	1
Arsenical 4	<1
Arsenical 5	1
Arsenical 6	1
Arsenical 7	<1
DHFR inhibitor 2	1
DHFR inhibitor 3	1
DHFR inhibitor 4	4
Guanylhydrazone B3	1
MDL 27695	<1
Nitromidazole 1	<1
Nitromidazole 2	<1
Purine nucleoside 3	1
Purine nucleoside 4	4
Purine nucleoside 5	2

[a] Ratio of median cyst count in the C/S group to that in the treated group.
[b] Median cyst count in the C/S group was 10^7 to 10^8/lung.

comparing *P. carinii* cyst or nucleus counts in the drug treatment groups with those in the C/S group in the same experiment. Such an approach allowed the activity of each candidate compound to be expressed in relation to its own control. Median counts were used rather than mean counts because they more accurately represented skewed data. The median cyst or nucleus count of the C/S group was divided by the median cyst or nucleus count of each drug treatment group, and the ratio was used an indicator of anti-*P. carinii* activity. Thus, if the median cyst count of the C/S group were 4×10^8 per lung and the median cyst count of the drug treatment group were 2×10^6 per lung, the ratio of 200 would signify a 200-fold reduction in cyst count.

The following categories of activity were established on the basis of the fall in median *P. carinii* cyst or nucleus

counts: inactive (<5-fold reduction), slight activity, (5- to 9-fold reduction), moderate activity (10- to 99-fold reduction), marked activity (100- to 999-fold reduction), and very marked activity (≥1,000-fold reduction). This system was chosen to mimic quantitative bacterial or fungal cultures, with each category representing about 1 log unit difference in organism burden.

The criteria used to establish some of the categories were influenced by features of the experimental test system. For example, the fivefold decline in cyst or nucleus count was chosen as the threshold for drug efficacy to provide clear evidence of activity; a number of compounds caused slight changes in the organism burden which were inconsistent and hence difficult to interpret. The ≥1,000-fold fall in organism count was chosen as the category of maximal drug activity on the basis of a usual median cyst count in the C/S group of at least 10^8 per lung and a detection limit of 1.1×10^5 per lung. Since compounds showing this activity usually reduced the cyst count to 1.1×10^5 per lung, it was not possible to determine whether different ratios (e.g., 7,000 versus 3,000) represented true differences in drug efficacy or simply reflected higher cyst counts in the C/S groups. When the median cyst count in the C/S group was 10^7 to 10^8 per lung, drugs which lowered the cyst count to 1.1×10^5 per lung were classified as showing very marked activity.

Analysis of drug efficacy by nucleus quantitation raised the issue of establishing an additional anti-*P. carinii* activity because of the higher median nucleus counts in the C/S group (10^8 to 10^9 per lung). However, this was believed not to be needed because fewer studies involving nucleus quantitation were performed.

RESULTS

Drugs tested in the therapy of pneumocystosis. The drugs which have been studied in the treatment of *P. carinii* pneumonia in the rat model are listed in Table 1. Most of the agents are listed by their generic names. A few compounds are listed by their general class (e.g., purine nucleoside 1 and guanylhydrazone A1) to protect the confidentiality of proprietary information. Some drugs were tested at one dose, whereas others were tested at several doses; in the latter case, the range of doses has been given.

Classification of therapeutic activity by cyst counts. The classification of drugs in the treatment of pneumocystosis by the reduction of cyst counts is described in Table 2. The ratios listed here signify the maximal activity of each drug or combination of drugs. Sulfonamides were among the most active anti-*P. carinii* drugs tested (36, 39). Sulfadiazine, sulfadoxine, and SMX repeatedly showed marked or very marked activity in experiments performed months or years apart. The sulfone dapsone was studied in a more limited manner and produced somewhat less consistent results. In early studies, the highest dose of dapsone (125 mg/kg/day) exhibited only moderate anti-*P. carinii* activity; however, in later experiments, this dose showed very marked activity. The sulfonamides and sulfones were well tolerated by the rats.

The most detailed studies were of drug efficacy were performed with SMX. The standard dose of this compound used in studies of experimental pneumocystosis is 250 mg/kg/day (15, 23). Doses of 60 to 500 mg/kg/day all showed very marked anti-*P. carinii* activity, and thus there was no way to establish a dose-response curve (39). More recent experiments used SMX regimens of 0.3 to 15 mg/kg/day, and a dose-response effect was found (36).

DHFR inhibitors used alone were ineffective in the treatment of pneumocystosis. Although these agents were tested in a variety of combinations with sulfonamides and dapsone, no evidence of synergy was found. The classification of activity of these drug combinations listed in Table 2 represents the activity of the sulfonamide or sulfone component. Most experiments with the sulfonamides were performed with the higher (≥60-mg/kg/day) doses of these compounds, as described above. Studies with dapsone were performed as part of the initial evaluation of this drug; this accounts for the apparent disparity in the classification of the activity of dapsone used in combination with DHFR inhibitors compared with that of dapsone used alone. Overall, the data suggested that additional experiments involving different dose regimens of these agents were needed to address the question of drug synergism.

The toxicity of the DHFR inhibitors varied among the individual agents. TMP and diaveridine were well tolerated by the rats, whereas pyrimethamine and trimetrexate caused bone marrow suppression and other adverse reactions. The administration of folinic acid was helpful in protecting against the toxicity of the DHFR inhibitors and did not interfere with efficacy.

The diamidines and related cationic compounds constituted the other major group of highly active anti-*P. carinii* drugs (40). In contrast to the sulfonamides, these agents demonstrated a hierarchy of anti-*P. carinii* activity. The most active drugs included selected guanylhydrazone derivatives; diminazine, a diamidine; imidocarb, a carbanilide; and quinapyramine, an aminoquinaldine. The guanylhydrazone A compounds were synthesized by procedures of Ulrich et al. (35), whereas the guanylhydrazone B derivatives were prepared by Richard Sundberg, University of Virginia (33). Pentamidine and five other compounds exhibited moderate activity. The *cis* form of dimethylstamidine was slightly active, whereas the *trans* form of this compound and several other drugs showed no activity.

The diamidine derivatives presented problems of toxicity, product formulation, and limited supplies. These agents frequently caused serious adverse reactions at doses only slightly higher than those found to show activity against *P. carinii*. Some compounds had been prepared many years ago, so there was little current information about their antimicrobial or pharmacological properties. Other drugs represented newly synthesized compounds and were present in small amounts; since nothing was known about what dose or form of these preparations would be best for oral or parenteral administration, drug testing was largely a matter of trial and error. Despite these limitations, the diamidine derivatives were the most potent anti-*P. carinii* drugs (on a milligram-per-kilogram basis) tested in our animal model.

Six purine nucleoside derivatives were studied. These compounds were initially synthesized by Robert Klein and Brian Otter, Montefiore Medical Center, and later obtained through the AIDS Developmental Therapeutics Branch, National Institutes of Health. Synthesis of purine nucleosides is very difficult, and only two compounds, 9-deazainosine and purine nucleoside 1, were available in sufficient quantities for testing at more than one dose. 9-Deazainosine showed marked anti-*P. carinii* activity (36), and purine nucleosides 1 and 2 showed slight activity. These results, and the lack of serious systemic toxicity, suggested that the purine nucleosides were worthy of further testing.

A variety of nitrofuran and nitroimidazole derivatives were evaluated (38). The most effective drug was furazolidone, which demonstrated moderate anti-*P. carinii* activity

on repeated occasions over a dose range of 50 to 200 mg/kg/day. Nitrofurantoin and metronidazole showed slight activity at high doses, but this effect was not consistent and could not be improved by manipulations such as changes in the preparation, dose, or route of administration. None of the other compounds exhibited any activity against *P. carinii*.

Many antiparasitic agents were tested, but the results were generally disappointing. The polyamine inhibitors DFMO and MDL 27695, obtained from Peter McCann, Merrell-Dow, Cincinnati, Ohio, were administered alone and in combination with other agents, but they showed little anti-*P. carinii* activity; DFMO was tolerated poorly by the rats (40). Similar data were obtained with arsenical derivatives synthesized by Ernst Friedheim, Rockefeller University, and with antimonials. Dehydroemetine, a toxic antiamoebic drug obtained from the Centers for Disease Control, Atlanta, Ga., exhibited moderate activity at the 5-mg/kg/day dose in one experiment but no activity at 1 mg/kg/day in another experiment; further studies with this compound are planned.

Antifungal drugs, administered alone or in combination, were ineffective in the therapy of pneumocystosis. Similar results were found with a series of antibiotics. We were especially interested in studying erythromycin because of anecdotal reports of its activity against *P. carinii* in humans; however, our analysis of several different preparations and doses of this agent gave disappointing results.

Of the antiviral agents studied, amantadine showed slight anti-*P. carinii* activity but the dose had to be reduced from 100 to 50 mg/kg/day because of side effects in the rats. The anti-HIV compounds azidothymidine (AZT), dideoxyinosine, and inosine pranobex (Isoprinosine) were inactive. AZT was also administered with TMP-SMX but exhibited no influence on the activity of this drug combination (36).

Classification of therapeutic activity by nucleus counts. The data for classification by nucleus counts are summarized in Table 3. In general, the pattern of anti-*P. carinii* drug activity as judged by nucleus counts was similar to that determined by cyst counts; sulfonamides and diamidines were the most active groups of compounds. The major difference was in the magnitude of the changes with nuclei counts. Drugs which lowered the cyst count by 1,000 to 10,000 frequently lowered the nucleus count by more than 10,000. This probably reflected the fact that nucleus counts were usually 10-fold higher than cyst counts; however, a greater sensitivity of *P. carinii* nuclei than cysts to some drugs could not be ruled out. The practical result of classifying drugs on the basis of changes in the nucleus counts was to increase the level of activity of some agents (e.g., pentamidine and amicarbalide).

Prophylaxis studies. Only three experiments investigating the efficacy of antimicrobial drugs in the prevention of pneumocystosis were performed. The compounds used in these studies and classification of their anti-*P. carinii* activity are presented in Tables 4 and 5, respectively. Most of the attention was devoted to antifolate drugs. SMX was highly effective as a prophylactic agent when administered alone over a broad dose range, as judged by the reduction in cyst counts. No evidence of synergy was found when the DHFR inhibitors were combined with SMX. Pyrimethamine and TMP both exhibited anti-*P. carinii* activity when used alone in one experiment, but this effect could not be reproduced in another experiment.

Of the other drugs tested, diminazene showed activity at a dose of 5 mg/kg/day subcutaneously but not when given less frequently or at a lower dose. Pentamidine and furazolidone were tested at only one dose.

TABLE 3. Classification of drugs in the treatment of *P. carinii* pneumonia by nucleus counts

Drug classification	Ratio[a]
Very marked activity	
Dapsone	2,000
Dapsone + pyrimethamine	2,000
Imidocarb	3,600
Pentamidine	25,000
Quinapyramine	30,000
Sulfadiazine	9,300
Sulfadiazine + diaveridine	9,300
Sulfadiazine + pyrimethamine	73,000
Sulfadiazine + TMP	9,300
Sulfadoxine	25,000
Sulfadoxine + pyrimethamine	73,000
SMX	15,000
SMX + Diaveridine	3,270
SMX + pyrimethamine	73,000
SMX + TMP	15,000
SMX + DHFR inhibitor 1	2,700
Marked activity	
Amicarbalide	491
Diminazine	714
Sulfadoxine + TMP	884
Moderate activity	
Isometamidium	37
Pyrimethamine	10
Slight activity	
Dapsone + diaveridine	5
Dapsone + TMP	5
DFMO	7
DFMO + diminazine	6
DFMO + pentamidine	9
Guanylhydrazone	8
No activity	
Ampicillin	<1
Astiban	2
Chloramphenicol	<1
Dehydroemetine	3
Diaveridine	<1
Ethidium bromide	3
Gentamicin	<1
Pentostam	1
Spectinomycin	<1
Spiramycin	1
TMP	<1

[a] Ratio of median nucleus count in the C/S group to that in the treated group.

DISCUSSION

The immunosuppressed rat model has proven to be a highly reliable system for studying the activity of antimicrobial compounds against *P. carinii*. Evaluation of drug efficacy has involved the microscopic determination of the extent of pneumocystosis in treatment groups compared with that in controls. This has been accomplished by the use of lung histologic scoring systems and by procedures for counting different developmental stages of *P. carinii* in lung homogenates or imprint smears. Data analysis has been performed with and without the aid of a variety of statistical

TABLE 4. Drugs evaluated in the prevention of *P. carinii* pneumonia[a]

Drug	Dose regimen (mg/kg/day)
Diminazine	1 (s.c.)–5 (s.c. 3–7 days/wk)
Furazolidone	50 (p.o. 5 days/wk)
Pentamidine	5 (s.c. 2 days/wk)
Pyrimethamine	3–4.5 (p.o.)
SMX	3–60 (p.o.)
Trimetrexate	3–4.5 (s.c.)
TMP	3–50 (p.o.)
SMX + Pyrimethamine	15 (p.o.) + 3 (p.o.)
SMX + Trimetrexate	15 (p.o.) + 3 (p.o.)
SMX + TMP	3–15 (p.o.) + 0.6–3 (p.o.)

[a] Rats given regimens containing pyrimethamine and trimetrexate were also given folinic acid (7 to 15 mg/kg per day s.c.).

techniques. All of these approaches have yielded valuable information; however, they have not provided a common framework to directly compare results.

In the present study, we have developed a simple, quantitative system for expressing the *P. carinii* organism burden in infected rats and the magnitude of the reduction which occurs with therapy. This was made possible by the consistency of our animal model and the large data base accumulated in testing antimicrobial drugs. Cyst counts were used more frequently than nucleus counts to assess drug efficacy because they were easier to perform. Median counts were used rather than mean counts because of the skewed data distribution which frequently occurred with drug treatment.

The central feature of the system involved calculation of the ratio of the median organism count of the control group to that of the drug treatment in the same experiment, with the result being taken as an expression of anti-*P. carinii* activity. This allowed drugs studied in experiments performed at different times to be compared with each other. Judging from results obtained with agents such as SMX over the past 7 years, the system has proven to be highly reproducible in the evaluation of the therapeutic activity of antimicrobial compounds; however, further studies are necessary before conclusions can be drawn about the value of the system in assessing the prophylactic abilities of these agents.

TABLE 5. Classification of drugs in the prevention of *P. carinii* pneumonia by cyst counts

Drug classification	Ratio[a]
Very marked activity	
SMX	6,300
SMX + Pyrimethamine	6,300
SMX + Trimetrexate	6,300
SMX + TMP	6,300
Marked activity	
Pyrimethamine	168
Moderate activity	
Diminazene	23
TMP	16
Slight activity	
Pentamidine	5
No activity	
Furazolidone	1
Trimetrexate	1

[a] Ratio of median cyst count in the C/S group to that in the treated group.

The classification system for drug activity was modeled after the approach used for quantitative microbial cultures. This provided clear categories of anti-*P. carinii* activity which followed a logarithmic scale and lessened the need for statistical analysis to demonstrate drug efficacy. Classes of drugs as well as individual members of a drug class could be distinguished from each other, and studies of dose-response curves were enhanced. *P. carinii* cyst and nucleus counts showed a similar pattern of response to therapy, although there were differences in the magnitude of this response. We believe that cyst quantitation can be used to screen most drugs for anti-*P. carinii* activity; however, with compounds (e.g., β-glucan inhibitors) which act on selective developmental stages of *P. carinii* (29), both cyst and nucleus counts are needed to get a complete picture of drug effects on the organism.

The classification system presented challenges for data interpretation at both ends of the spectrum of drug activity. The long list of compounds classified as inactive suggested that the level of reduction in *P. carinii* cyst or nucleus counts established for minimal activity was effective in screening out the minor effects of drugs. We believe that the benefits of this standard far outweighed any loss of sensitivity. Quantitative differences in the reduction in cyst or nucleus counts among drugs classified as very markedly active could not be accurately interpreted because of the inability of our system to detect fewer than 1.1×10^5 organisms per lung. This emphasized the need for more sensitive techniques to quantitate low levels of infection.

In recent years, there has been increased interest in developing new anti-*P. carinii* drugs. However, the published literature does not give an accurate estimate of the number of compounds which have been studied because most of these reports have emphasized agents which have exhibited activity against *P. carinii*. The present study has provided a comprehensive review of drugs evaluated by one group of investigators. A broad array of compounds was investigated, ranging from drugs in clinical use to newly synthesized agents; this illustrates that selection of drugs for testing in the rat model of pneumocystosis is often empirical, owing to the lack of reliable in vitro test systems and readily identifiable molecular or biochemical targets.

Our drug-screening efforts have focused on several classes of compounds. Analysis of antifolate drugs revealed that anti-*P. carinii* activity is a general property of sulfonamides and also probably of sulfones; DHFR inhibitors exhibited little consistent activity against *P. carinii* and did not enhance the efficacy of the sulfonamides (39). There are several possible mechanisms for this lack of activity of DHFR inhibitors in the rat model, which are examined in the accompanying paper (36). Studies are currently under way to examine the use of low doses of sulfonamides and dapsone with different doses of DHFR inhibitors to see whether synergistic combinations can be found.

Another major area of interest involved the diamidines and related compounds, which were originally developed as antitrypanosomal agents (40). Although these drugs were toxic, they were highly effective in the therapy of pneumocystosis. Many of the diamidine derivatives have been investigated as anti-*P. carinii* drugs (21, 34), but little attention has been paid to the guanylhydrazones. The availability of structure-activity data concerning the effects of guanylhydrazones on trypanosomes (33) should be of help in developing anti-*P. carinii* drugs. Our experience with gua-

nylhydrazone derivatives in the rat model of pneumocystosis will be reported in detail in a separate publication.

There has been growing interest in developing purine nucleosides as antiprotozoal drugs (25). 9-Deazainosione has shown promising activity against *P. carinii* (32, 36), and our limited analysis of other purine nucleoside analogs suggests that anti-*P. carinii* activity might be a general property of this class of compounds. We believe that purine nucleosides deserve in-depth analysis as potential anti-*P. carinii* agents.

Of the nitrofurans, furazolidone was an attractive compound because it is an oral preparation already licensed for clinical use. Furazolidone exhibited anti-*P. carinii* activity in our rat model at doses far higher than those used in humans; therefore, it is unlikely that furazolidone will ever be studied in clinical trials (38). Nevertheless, since thousands of nitrofuran derivatives have been synthesized, further evaluation of these compounds might be productive.

With few exceptions, our studies of other antiparasitic, antifungal, antibacterial, and antiviral drugs for activity against *P. carinii* have been disappointing. Some of this work has involved the administration of combinations of drugs. Perhaps the most interesting finding was that the anti-HIV drug AZT had no influence on the effectiveness of TMP-SMX against *P. carinii* (36). AZT is commonly administered to HIV patients along with antimicrobial drugs to treat or prevent opportunistic infections; our studies in the rat model provide experimental support for this practice.

In conclusion, this study has presented a detailed review of our experience of testing drugs in the rat model of pneumocystosis and proposed a new method of classifying their activity. This information should be helpful in developing new anti-*P. carinii* compounds and in standardizing procedures for their evaluation.

ACKNOWLEDGMENTS

This study was supported by the Medical Research Service of the Department of Veterans Affairs and by Public Health Service contract NO1-AI-72646 and grants AI 25897, HL 46653, AI 31702, and AI 32899 from the National Institutes of Health.

REFERENCES

1. **Bartlett, M. S., S. F. Queener, R. R. Tidwell, W. K. Milhous, J. D. Berman, W. Y. Ellis, and J. W. Smith.** 1991. 8-Aminoquinolines from Walter Reed Army Institute for Research for treatment and prophylaxis of *Pneumocystis carinii* in rat models. Antimicrob. Agents Chemother. **35:**277–282.

1a. **Bernard, E., and D. Armstrong.** Personal communication.

2. **Brun-Pascaud, M., M. Fay, M. Zhong, J. Bauchet, A. Dux-Guyot, and J. J. Poeidalo.** 1992. Use of fluoroquinolones for prophylaxis of murine *Pneumocystis carinii* pneumonia. Antimicrob. Agents Chemother. **36:**470–472.

3. **Clarkson, A. B., Jr., D. E. Williams, and C. Rosenberg.** 1988. Efficacy of DL-α-difluoromethylornithine in a rat model of *Pneumocystis carinii* pneumonia. Antimicrob. Agents Chemother. **32:**1158–1163.

4. **Clarkson, A. B., Jr., M. Saric, and R. W. Grady.** 1990. Deferoxamine and eflornithine (DL-α-difluoromethylornithine) in a rat model of *Pneumocystis carinii* pneumonia. Antimicrob. Agents Chemother. **34:**1833–1835.

5. **Cushion, M. T., J. J. Ruffolo, and P. D. Walzer.** 1988. Analysis of the developmental stages of *Pneumocystis carinii* in vitro. Lab. Invest. **58:**324–331.

6. **Cushion, M. T., D. Stanforth, J. J. Ruffolo, M. J. Linke, and P. D. Walzer.** 1985. Method of testing the susceptibility of *Pneumocystis carinii* to antimicrobial agents in vitro. Antimicrob. Agents Chemother. **28:**796–801.

7. **D'Antonio, R. G., D. B. Johnson, R. E. Winn, A. F. Van Dellen, and M. E. Evans.** 1986. Effect of folinic acid on the capacity of trimethoprim-sulfamethoxazole to prevent and treat *Pneumocystis carinii* pneumonia in rats. Antimicrob. Agents Chemother. **29:**327–329.

8. **Debs, R. J., W. Blumenfeld, E. N. Brunette, R. M. Straubinger, A. B. Montgomery, E. Lin, N. Agabian, and D. Papahadjopoulos.** 1987. Successful treatment with aerosolized pentamidine of *Pneumocystis carinii* pneumonia in rats. Antimicrob. Agents Chemother. **31:**37–41.

9. **Frenkel, J. K., J. T. Good, and J. A. Schultz.** 1966. Latent Pneumocystis infection of rats, relapse, and chemotherapy. Lab. Invest. **15:**1559–1577.

10. **Gigliotti, F., and W. T. Hughes.** 1988. Passive immunoprophylaxis with specific monoclonal antibody confers partial protection against *Pneumocystis carinii* pneumonitis in animal models. J. Clin. Invest. **81:**1666–1668.

11. **Girard, P. M., M. Brun-Pascaud, R. Farinotti, L. Tamisier, and S. Kernbaum.** 1987. Pentamidine aerosol in prophylaxis and treatment of murine *Pneumocystis carinii* pneumonia. Antimicrob. Agents Chemother. **31:**978–981.

12. **Hughes, W. T.** 1987. *Pneumocystis carinii* pneumonitis, vol. 2, p. 74–75. CRC Press, Inc., Boca Raton, Fla.

13. **Hughes, W. T.** 1991. Macrolide-antifol synergism in anti-*Pneumocystis carinii* therapeutics. J. Protozool. **38:**160S.

14. **Hughes, W. T., V. L. Gray, W. E. Gutteridge, V. S. Latter, and M. Pudney.** 1990. Efficacy of a hydroxynaphthoquinone, 566C80, in experimental *Pneumocystis carinii* pneumonitis. Antimicrob. Agents Chemother. **34:**225–228.

15. **Hughes, W. T., P. C. McNabb, and T. D. Makres.** 1974. Efficacy of trimethoprim and sulfamethoxazole in the prevention and treatment of *Pneumocystis carinii* pneumonitis. Antimicrob. Agents Chemother. **5:**289–293.

16. **Hughes, W. T., and B. L. Smith.** 1983. Intermittent chemoprophylaxis for *Pneumocystis carinii* pneumonia. Antimicrob. Agents Chemother. **24:**300–301.

17. **Hughes, W. T., and B. L. Smith.** 1984. Efficacy of diaminodiphenylsulfone and other drugs in murine *Pneumocystis carinii* pneumonitis. Antimicrob. Agents Chemother. **26:**436–440.

18. **Hughes, W. T., B. L. Smith, and D. P. Jacobus.** 1986. Successful treatment and prevention of murine *Pneumocystis carinii* pneumonitis with 4,4′-sulfonylbisformanilide. Antimicrob. Agents Chemother. **29:**509–510.

19. **Hughes, W. T., and B. L. Smith-McCain.** 1986. Effects of sulfonylurea compounds on *Pneumocystis carinii*. J. Infect. Dis. **153:**944–947.

20. **Hussain, Z., M. L. Carlson, I. D. Craig, and R. Lannigan.** 1985. Efficacy of tetroxoprim/sulfadiazone in the treatment of *Pneumocystis carinii* in rats. J. Antimicrob. Chemother. **15:**575–578.

21. **Jones, S. K., J. E. Hall, M. A. Allen, S. D. Morrison, K. A. Ohemeng, V. V. Reddy, J. D. Geratz, and R. R. Tidwell.** 1990. Novel pentamidine analogs in the treatment of experiment *Pneumocystis carinii* pneumonia. Antimicrob. Agents Chemother. **34:**1026–1030.

22. **Kim, C. K., J. M. Foy, M. T. Cushion, D. Stanforth, M. J. Linke, H. L. Hendrix, and P. D. Walzer.** 1987. Comparison of histologic and quantitative techniques in evaluation of therapy for experimental *Pneumocystis carinii* pneumonia. Antimicrob. Agents Chemother. **31:**197–201.

23. **Kluge, R. M., D. M. Spaulding, and J. A. Spain.** 1978. Combination of pentamidine and trimethoprim-sulfamethoxazole in the therapy of *Pneumocystis carinii* pneumonia in rats. Antimicrob. Agents Chemother. **13:**975–978.

24. **Kovacs, J. A., C. J. Allegra, S. Kennedy, J. C. Swan, J. Drake, J. E. Parrillo, B. Chabner, and H. Masur.** 1988. Efficacy of trimetrexate, a potent lipid-soluble antifolate, in the treatment of rodent *Pneumocystis carinii* pneumonia. Am. J. Trop. Med. Hyg. **39:**491–496.

25. **Marr, J. J., R. L. Berens, N. K. Cohn, D. J. Nelson, and R. S. Klein.** 1984. Biological action of inosine analogs in *Leishmania* and *Trypanosoma* spp. Antimicrob. Agents Chemother. **25:**292–295.

26. **Matsumoto, Y., M. Yamada, and T. Amagai.** 1991. Yeast glucan of *Pneumocystis carinii* cyst wall: an excellent target for chemotherapy. J. Protozool. **38:**6S–7S.

27. **Queener, S. F., M. S. Bartlett, M. A. Jay, M. M. Durkin, and J. W. Smith.** 1987. Activity of lipid-soluble inhibitors of dihydrofolate reductase against *Pneumocystis carinii* in culture and in a rat model of infection. Antimicrob. Agents Chemother. **31:**1323–1327.
28. **Queener, S. F., M. S. Bartlett, J. D. Richardson, M. M. Durkin, M. A. Jay, and J. W. Smith.** 1988. Activity of clindamycin with primaquine against *Pneumocystis carinii* in vitro and in vivo. Antimicrob. Agents Chemother. **32:**807–813.
29. **Schmatz, D. M., M. Powles, D. McFadden, and L. Pittarelli.** 1991. Treatment and prevention of *Pneumocystis carinii* pneumonia and further elucidation of the *Pneumocystis carinii* life cycle with 1,3-β-glucan synthesis inhibitor L-671,329. J. Protozool. **38:**151S–153S.
30. **Schmatz, D. M., M. A. Romancheck, L. A. Pittarelli, R. E. Schwartz, R. A. Fromtling, K. H. Nollstadt, F. L. Vanmiddlesworth, K. E. Wilson, and M. J. Turner.** 1990. Treatment of *Pneumocystis carinii* with 1,3-β-glucan synthesis inhibitors. Proc. Natl. Acad. Sci. USA **87:**5950–5945.
31. **Shear, H. L., G. Valladares, and M. A. Narachi.** 1990. Enhanced treatment of *Pneumocystis carinii* pneumonia in rats with interferon-γ and reduced doses of trimethoprim/sulfmethoxazole. J. Acquired Immune Defic. Syndr. **3:**943–948.
32. **Smith, J. W., M. S. Bartlett, S. F. Queener, M. M. Durkin, M. A. Jay, M. T. Hull, R. S. Klein, and J. J. Marr.** 1987. *Pneumocystis carinii* pneumonia therapy with 9-deazainosine in rats. Diagn. Microbiol. Infect. Dis. **7:**113–118.
33. **Sundberg, R. J., D. L. Dahlhausen, G. Manikumar, B. Mavunkel, A. Biswas, V. Srinivasan, H. A. Musallan, W. A. Reid Jr., and A. L. Ager.** 1990. Cationic antiprotozoal drugs. Trypanocidal activity of 2-(4′-formylphenyl)imidazo[1,2α]pyridinium guanylhydrazones and related derivatives of quaternary heteroatomic compounds. J. Med. Chem. **33:**298–307.
34. **Tidwell, R. R., S. K. Jones, J. D. Geratz, K. A. Ohemeng, M. Cory, and J. E. Hall.** 1990. Analogues of 1,5-bis(4-amidinophenoxy)pentane (pentamidine) in the treatment of experimental *Pneumocystis carinii* pneumonia. J. Med. Chem. **33:**1252–1257.
35. **Ulrich, P., and A. Cerami.** 1984. Trypanocidal 1,3 arylene diketone bis(guanylhydrazone)s. Structure-activity relationships among substituted and heterocyclic analogues. J. Med. Chem. **27:**35–40.
36. **Walzer, P. D., J. Foy, P. E. Steele, C. K. Kim, M. White, R. Klein, B. Otter, and C. Allegra.** 1992. Activities of antifolate, antiviral, and other drugs in the immunosuppressed rat model of *Pneumocystis carinii* pneumonia. Antimicrob. Agents Chemother. **36:**1935–1942.
37. **Walzer, P. D., C. K. Kim, and M. T. Cushion.** 1989. *Pneumocystis carinii*, p. 83–178. *In* P. D. Walzer and R. M. Gerter (ed.), Parasitic infections in the compromised host. Marcel Dekker, Inc., New York.
38. **Walzer, P. D., C. K. Kim, and J. Foy.** 1991. Furazolidone and nitrofurantoin in the treatment of experimental *Pneumocystis carinii* pneumonia. Antimicrob. Agents Chemother. **35:**158–163.
39. **Walzer, P. D., C. K. Kim, J. M. Foy, M. J. Linke, and M. T. Cushion.** 1988. Inhibitors of folic acid synthesis in the treatment of experimental *Pneumocystis carinii* pneumonia. Antimicrob. Agents Chemother. **32:**96–103.
40. **Walzer, P. D., C. K. Kim, J. M. Foy, M. J. Linke, and M. T. Cushion.** 1988. Cationic antitrypanosomal and other antimicrobial agents in the treatment of experimental *Pneumocystis carinii* pneumonia. Antimicrob. Agents Chemother. **32:**896–905.
41. **Walzer, P. D., R. D. Powell, K. Yoneda, M. E. Rutledge, and J. E. Milder.** 1980. Growth characteristics and pathogenesis of experimental *Pneumocystis carinii* pneumonia. Infect. Immun. **27:**929–937.
42. **Yamada, M., S. Takeuchi, T. Shiota, Y. Matsumoto, H. Yoshikawa, K. Okabayashi, T. Tegoshi, T. Yoshikawa, and Y. Yoshida.** 1985. Experimental studies on the chemoprophylaxis for *Pneumocystis carinii* pneumonia with intermittent administration of trimethoprim-sulfamethoxazole and pyrimethamine-sulfamonomethoxine. Jpn. J. Trop. Med. Hyg. **13:**287–294.
43. **Yoshida, Y., S. Takeuchi, K. Ogino, T. Ikai, and M. Yamada.** 1977. Studies on *Pneumocystis carinii* and *Pneumocystis carinii* pneumonia. III. Therapeutic experiment of the pneumonia with pyrimethamine + sulfamonomethoxine and trimethoprim + sulfamethoxazole. Jpn. J. Parasitol. **26:**367–375.