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Cationic Antitrypanosomal and Other Antimicrobial Agents in the Therapy of Experimental *Pneumocystis carinii* Pneumonia

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Cationic compounds used in the treatment of veterinary African trypanosomiasis have structural properties similar to those of pentamidine, which has been used in the therapy of human trypanosomiasis and infection with *Pneumocystis carinii*. We have compared the activities of these drugs and other antimicrobial agents in an immunosuppressed rat model of *P. carinii* pneumonia. Diminazene, imidocarb, amicarbalide, quinapyramine, and isometamidium showed efficacy greater than or equal to that of pentamidine in the therapy of *P. carinii* infection, whereas ethidium and methylglyoxal bis(guanylhydrazone) were only slightly active against the organism. Diminazene and pentamidine also exhibited comparable efficacy in *P. carinii* prophylaxis. α-Difluoromethylornithine (DFMO), a polyamine inhibitor, was ineffective therapy when used alone and did not improve the effectiveness of pentamidine or diminazene. Quinine, quinidine, quinacrine, chlorpromazine, spiramycin, Pentostam, Astiban, dehydroemetine, ampicillin, gentamicin, chloramphenicol, and spectinomycin also showed little or no activity against the organism. Thus, in this model anti-*P. carinii* activity appears to be a common property of veterinary cationic trypanocidal compounds. This should be important in studying structure-activity relationships and in developing new drugs for the treatment of *P. carinii* infection in humans.

Since the discovery of the acquired immunodeficiency syndrome (AIDS) in 1981 (13, 26), the incidence of *Pneumocystis carinii* pneumonia in this country has increased dramatically. *P. carinii* is the most frequent opportunistic pathogen in AIDS, occurring in over 60% of cases (10). The problems of treatment of *P. carinii* infection in AIDS (e.g., the high rate of relapse and adverse drug reactions) have emphasized the need to develop new forms of therapy (12, 14, 24, 39).

Drug development for *P. carinii* has generally been empiric because of a lack of knowledge about the metabolic pathways of the organism. An alternative approach would be to explore compounds which are related to drugs currently in use for the treatment of *P. carinii* infection. Pentamidine isethionate, one of the principal anti-*P. carinii* drugs, is a diamidine originally introduced for human clinical use over 40 years ago for the treatment of African trypanosomiasis (32). We wondered whether other old cationic quaternary ammonium compounds with structural properties similar to those of pentamidine, which have been used in the therapy of veterinary African trypanosomiasis, might have activity against *P. carinii*. Support for this hypothesis can be found in reports which have shown anti-*P. carinii* activity with hydroxystilbamidine, a diamidine, and with the structurally unrelated antitrypanosomal drugs α-difluromethylornithine (DFMO) and 9-deazinosine (4, 11, 28).

Experimental systems for anti-*P. carinii* drug evaluation have mainly consisted of immunosuppressed animals. Rats administered corticosteroids for 6 to 8 weeks spontaneously develop *P. carinii* pneumonia with histologic features identical to the human form of the disease; several drug studies have shown that the rat model is usually a reliable predictor of activity in humans (11, 15, 16, 23). Our laboratory has previously developed histologic and quantitative methods for measuring the severity of corticosteroid-induced *P. carinii* infection in rats and for evaluating the effects of treatment (7–9, 22, 42, 43). The present study was designed to compare pentamidine with cationic veterinary trypanocidal compounds, DFMO, and other antimicrobial drugs in the therapy of experimental *P. carinii* pneumonia.

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MATERIALS AND METHODS

Animal protocol. The basic experimental design, which has been described in detail in our earlier studies (22, 42, 43), involved the use of adult male Sprague-Dawley rats obtained from Harlan Industries (Madison, Wis.) and weighing about 250 g. The animals were housed in a conventional colony for a period of acclimatization and then placed on the immunosuppressive regimen of 4 mg of methylprednisolone (Depo-Medrol; The Upjohn Co., Kalamazoo, Mich.) injected subcutaneously once weekly, a low (8%) protein diet (Bioserv, Frenchtown, N.J.), and tetracycline powder (Polyotic; American Cyanamid, Wayne, N.J.) (1 mg/ml) in the drinking water to induce *P. carinii* pneumonia. The rats were weighed at regular intervals, and sentinel animals were sacrificed at different time points to monitor the development of disease. After 5 to 6.5 weeks, when the infection had become moderately advanced, the rats were randomly divided into different treatment groups of about 16 animals each. Anti-*P. carinii* drugs were administered for 3 weeks, during which time all rats remained on the immunosuppressive regimen; control animals on this steroid protocol received no treatment. (Pilot experiments had revealed no differences in the extent of *P. carinii* infection in immunosuppressed rats which received no therapy or among animals which received a placebo.) At the end of the period of therapy, the rats were sacrificed by an overdose of halothane anesthesia.

In selected experiments, some drugs (e.g., diamidines) were evaluated in the prophylaxis of *P. carinii* infection. These agents were administered throughout the 9-week period of corticosteroid immunosuppressive regimen, after

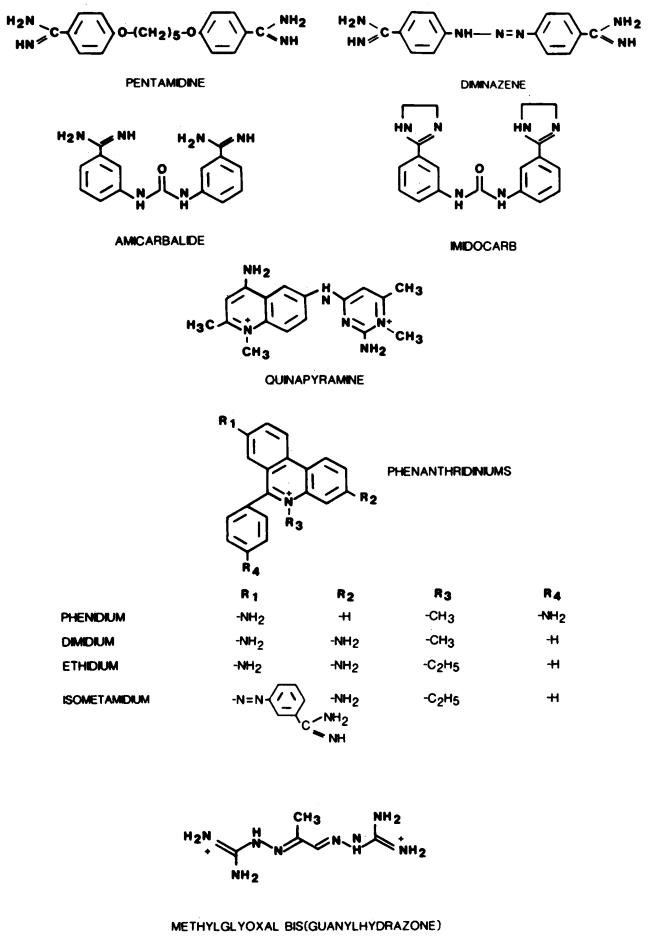

FIG. 1. Structural formulas of cationic antitrypanosomal drugs.

which time the rats were sacrificed and their lungs were examined in the same manner used in the treatment studies.

Drugs. The cationic antitrypanosomal drugs were obtained from the following sources: pentamidine isethionate from Lypho Med, Inc., Melrose Park, Ill.; diminazene aceturate from Sigma Chemical Co., St. Louis, Mo.; imidocarb dihydrochloride as a gift from Edmund Wise, Burroughs Wellcome Co., Research Triangle Park, N.C.; amicarbalide isethionate as a gift from John Dowding, May and Baker, Ltd. Dagenham, England; quinapyramine methylsulfate as a gift from M. Joseph, Imperial Chemical Industries, Ltd, Macclesfield, Cheshire, England; isometamidium chloride as a gift from May and Baker; ethidium bromide from Sigma; methylglyoxal bis(guanylhydrazone) dihydrochloride (MGBG) from Sigma. The structural formulas of these compounds have been presented in Fig. 1.

Other drugs included DFMO obtained as a gift from Peter McCann, Merrell Dow Research Institute, Cincinnati, Ohio; quinine sulfate from Sigma; quinidine sulfate from Sigma; quinacrine dihydrochloride from Sigma; chlorpromazine hydrochloride from Sigma; spiramycin as a gift from Rhone Poulenc, Inc., Monmouth Junction, J.J.; Pentostam (Wellcome Foundation, Ltd., London, England) from the Centers for Disease Control (CDC), Atlanta, Ga.; Astiban (Hoffmann-La Roche, Basel, Switzerland) from CDC; dehydroemetine (Hoffmann-La Roche) from CDC; ampicillin sodium from Wyeth Labs, Philadelphia, Pa.; gentamicin sulfate from Schering Corp., Kenilworth, N.J.; chloramphenicol sodium succinate from Parke-Davis, Morris Plains, N.J.; and spectinomycin hydrochloride from The Upjohn Co., Kalamazoo, Mich.

The compounds were usually diluted in water and administered orally (p.o.) by gavage or parenterally by subcutaneous (s.c.), intramuscular (i.m.), or intraperitoneal (i.p.) injection. In some instances, preliminary experiments were conducted to determine the optimal dose schedule and route of administration. All drugs were administered on a milligram-per-kilogram basis as a single dose, which was based on the average weight of the rats (about 150 g) at the beginning of treatment and remained the same throughout the 3-week course of therapy. The rats were examined daily for signs of drug toxicity. When adverse reactions were detected, the drug was held until the animals recovered and an alternative dose schedule or route of administration was instituted.

Assessment of treatment of *P. carinii* infection. Evaluation of drug therapy was complicated by the fact that animals on the immunosuppressive regimen to induce *P. carinii* infection were very susceptible to the toxic effects of anti-*P. carinii* drugs and to other opportunistic infections; thus, rats frequently died before completing a full 3-week course of therapy. We decided to base the assessment of drug efficacy on the extent of *P. carinii* infection in the lungs rather than on animal survival (22, 42). *P. carinii* pneumonia in drug-treated rats was compared with that in controls in the same study; when a compound was used in more than one study, the control groups were pooled. Our preliminary data revealed that 10 days were sufficient to observe a response to treatment; unless otherwise specified, only rats which received ≥10 days of anti-*P. carinii* therapy were included in the data analysis.

P. carinii infection in lungs was analyzed by histologic and quantitative techniques described in detail previously (7–9, 22, 42, 43). At death or time of sacrifice, the left lung of each rat was removed, infused with 4% formaldehyde through the bronchus until fully expanded, and fixed for histologic preparation. Three horizontal sections (one each from the upper, middle, and lower portions) of the lung were stained with hematoxylin and eosin, which provided a general view of lung structures, and with Grocott methenamine silver, which selectively stained the wall of *P. carinii* cysts. The lung sections were coded and read blindly. The following scoring system was used to assess the extent of *P. carinii* infection, based on the proportion of alveoli involved: 0, no infection; 0.5, minimal (<1% alveoli); 1+, light (1 to 25% alveoli); 2+, moderate (25 to 50% alveoli); 3+, severe (50 to 75% alveoli); 4+, very severe (>75% alveoli).

The right lung was removed and used for organism quantitation. The organ was weighed and homogenized in a stomacher (Tekmar, Cincinnati, Ohio), and freshly prepared samples were air dried and stained with two types of stains: cresyl echt violet, which selectively stains *P. carinii* cysts, and Diff Quik, which stains the nuclei of cysts, trophozoites, and intermediate forms. Specimens were read in a blinded manner. The lower limit of detection is 1.47×10^5 organisms per lung.

We found in our earlier studies that the histologic scoring system and quantitation procedures provided comparable evaluation of drug treatment of *P. carinii* infection (22, 42). In the present study, histopathologic examination was performed on all rat lungs, whereas the quantitation techniques were performed on about eight animal specimens in each treatment group. The changes in cyst and nuclei counts after *P. carinii* treatment were similar, and thus only the cyst

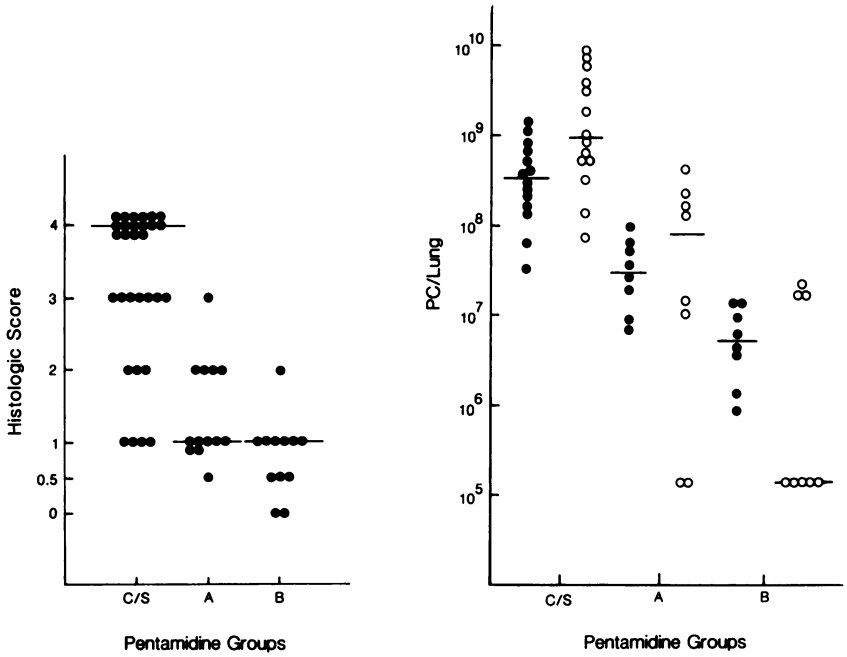

FIG. 2. Treatment of *P. carinii* infection with pentamidine as assessed by histologic score (left) and quantitation of *P. carinii* cysts (●) and nuclei (○) (right). The following pentamidine dose regimens were used: A, 10 mg/kg t.i.w. i.m.; B, 20 mg/kg t.i.w. i.m. C/S, Control steroid group. Horizontal bars represent median values.

results have been presented in detail here; nuclei data have been included on a selective basis to illustrate the changes resulting from treatment.

RESULTS

Diamidines as therapeutic agents. Pentamidine in doses of 10 mg/kg (group A) and 20 mg/kg (group B) three times weekly (t.i.w.) was moderately effective in the treatment of *P. carinii* infection (Fig. 2). The median histologic score in the control steroid group fell from 4+ to 1+ in the treated groups. Dose-related effects of pentamidine were more noticable in the quantitation studies in which median *P. carinii* cyst counts fell from 3.46×10^8 per lung in the control steroid group to 3.36×10^7 per lung in group A and 5.31×10^6 per lung in group B rats; similar changes occurred in nuclei counts. The doses of pentamidine used here were similar to those used by other investigators (11, 15, 23). The i.m. route of administration was used, because in our preliminary studies, pentamidine given s.c. caused considerable pain and necrosis at the injection sites.

Diminazene exhibited activity against *P. carinii* similar to that achieved with pentamidine (Fig. 3). Increasing doses of diminazene resulted in a progressive fall in the histologic score of 4+ in control steroid group rats to 0.5+ in animals treated with 10 mg/kg per day (group C); similar effects were noted in the *P. carinii* cyst and nuclei counts. These doses of diminazene were well tolerated, with no local or systemic reactions being noted. However, diminazene at a dose of 20 mg/kg per day was markedly toxic to the rats and had to be reduced to 15 mg/kg per day (group D). Adverse reactions ranged from marked lethargy and general clinical deterioration to death. This higher dose of diminazene did not improve therapeutic efficacy, as judged by histologic or quantitative criteria.

DFMO and the diamidines. DFMO was used alone and in combination with pentamidine or diminazene (Fig. 4). DFMO administered as a 4% solution in the drinking water caused severe diarrhea in the rats, leading to debilitation and early death; even with reduction of the dose to a 2% solution, there was still some morbidity and mortality. For the purposes of data analysis, results from rats on the different dose regimens of DFMO have been combined. DFMO used alone (group A) exhibited little activity against *P. carinii* by histopathologic examination or organism quantitation. Rats administered DFMO and different doses of pentamidine (group B) had a median histologic score of 1+ compared with 4+ in the control steroid group; reductions were also noted in *P. carinii* cyst and nuclei counts. However, these results were not better than those achieved when pentamidine was used alone (Fig. 2). Similarly, the therapeutic efficacy of DFMO combined with diminazene (group C) was no better than that of diminazene alone (Fig. 3).

Diamidines as prophylactic agents. Pentamidine and diminazene administered throughout the entire period of the corticosteroid immunosuppressive regimen were compared for their ability to prevent the development of *P. carinii* pneumonia (Fig. 5). Rats administered pentamidine in a dose of 5 mg/kg twice weekly i.m. (group A) had a median histologic score of 2+, a cyst count of 5.11×10^7 per lung, and a nuclei count of 2.38×10^8 per lung compared with corresponding values of 4+, 2.95×10^8 per lung, and 3.61×10^9 per lung in the control steroid group. Diminazene was given in dose schedules of 1 mg/kg t.i.w. s.c. (group B), 5 mg/kg t.i.w. s.c. (group C), and 5 mg/kg per day s.c. (group D). As judged by both histologic and quantitative criteria, diminazene demonstrated anti-*P. carinii* activity which was related to dose and frequency of administration. The prolonged administration of pentamidine and diminazene was well tolerated by the rats, with all groups showing median survival times of 63 days.

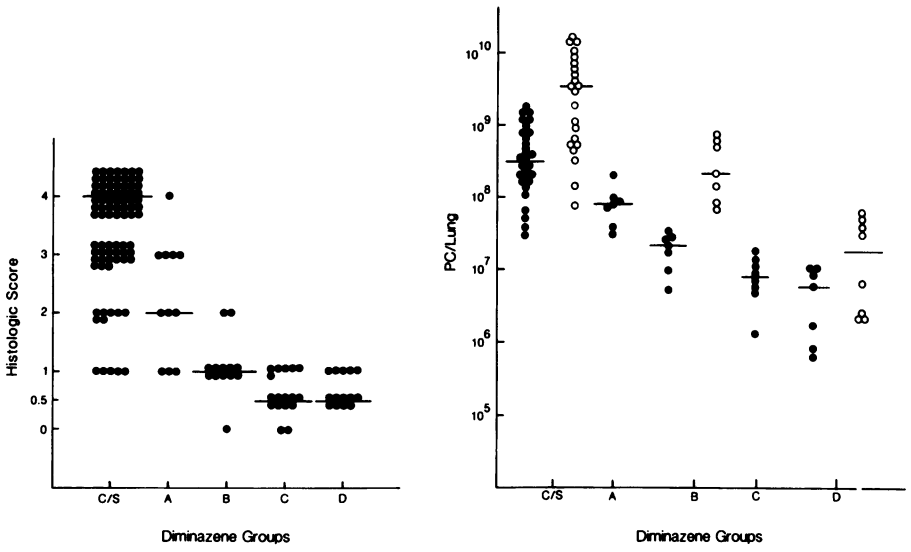

FIG. 3. Treatment of *P. carinii* infection with diminazene as assessed by histologic score (left) and quantitation of *P. carinii* cysts (●) and nuclei (○) (right). The following diminazene dose regimens were used: A, 2.5 mg/kg per day s.c.; B, 5 mg/kg per day s.c.; C, 10 mg/kg per day s.c.; D, 20 → 15 mg/kg per day s.c. C/S, Control steroid group.

Carbanilides. Imidocarb was very active in the treatment of *P. carinii* infection (Fig. 6). The median histologic score ranged from 1+ in animals treated with a dose of 2.5 mg/kg per day (group A) to 0 in animals treated with 10 mg/kg per day (group C). *P. carinii* cyst counts fell from 3.93×10^8 per lung in the control steroid group to 1.21×10^7 per lung in group A and 7.35×10^5 per lung in group C rats; an even greater decline was noted in nuclei counts. These doses of imidocarb produced no discernable toxic effects in the animals. Doses of 15 mg/kg per day (group D) and 25 mg/kg per day (group E) were poorly tolerated and had to be reduced; adverse reactions ranged from general clinical deterioration to early death. The therapeutic efficacy of imidocarb in groups D and E was not enhanced.

Amicarbalide exhibited moderate dose-related activity against *P. carinii* (Fig. 7). The median histologic score fell from 4+ in the control steroid group to 3+ in animals treated with a dose of 1.5 mg/kg per day (group A) and to 1+ in animals treated with a dose of 2.5 mg/kg per day (group C). *P. carinii* cyst counts declined from 5.82×10^8 per lung in control steroid group rats to 1.83×10^7 per lung in group 5C animals. These doses of amicarbalide were well tolerated. Administration of amicarbalide in a dose of 10 mg/kg per day (group D) had to be progressively reduced to 5 mg every

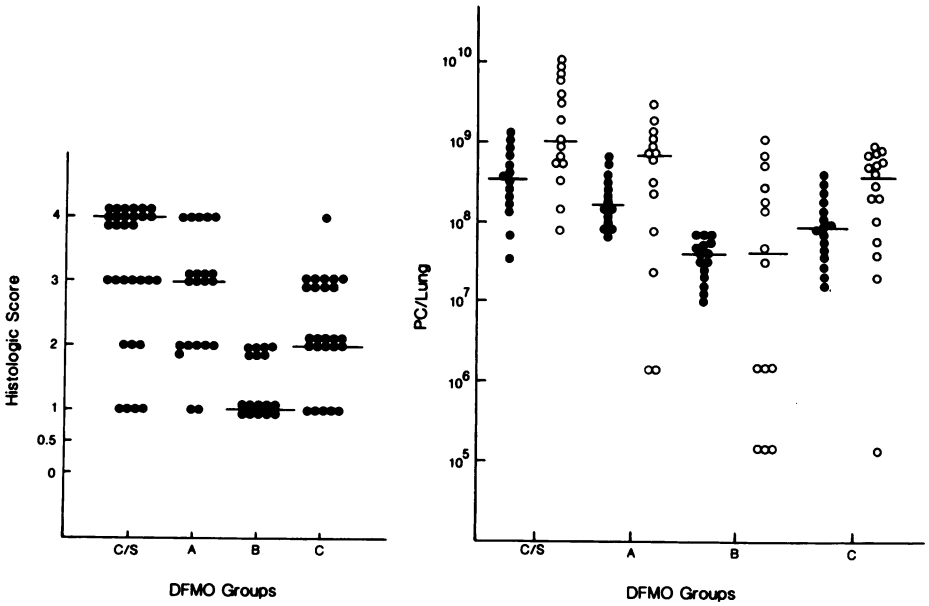

FIG. 4. Treatment of *P. carinii* infection with DFMO alone and in combination with pentamidine or diminazene as assessed by histologic score (left) and quantitation of *P. carinii* cysts (●) and nuclei (○) (right). The following DFMO dose regimens were used: A, 4% → 2% oral solution; B, DFMO 4% → 2% oral solution + pentamidine (10 or 20 mg/kg t.i.w. i.m.); C, DFMO 4% → 2% oral solution + diaminazene (5 or 2.5 mg/kg per day s.c.). C/S, Control steroid group.

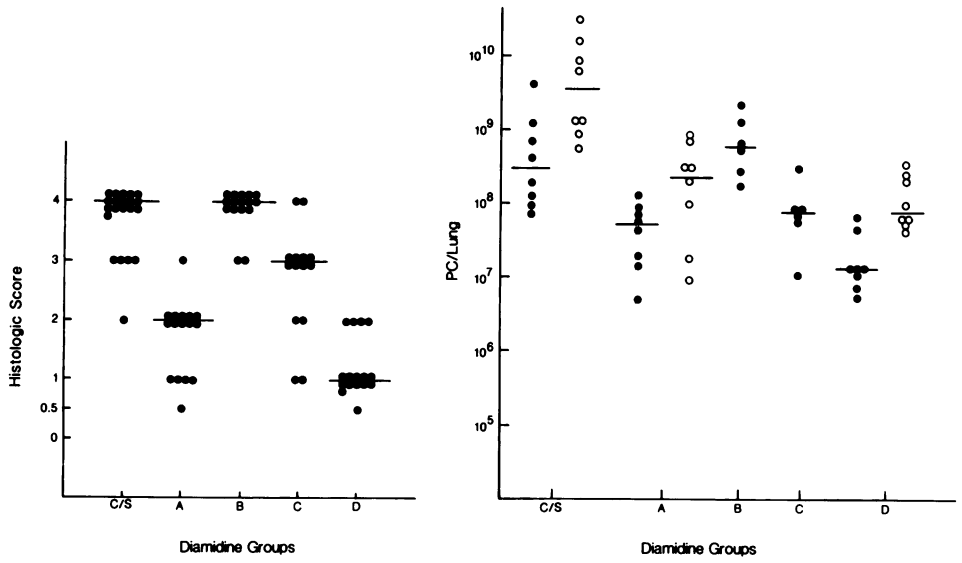

FIG. 5. Prophylaxis of *P. carinii* infection with pentamidine and diminazene as assessed by histologic score (left) and quantitation of *P. carinii* cysts (●) and nuclei (○) (right). The following diamidine regimens were used: A, pentamidine (5 mg/kg b.i.w. i.m.); B, diminazene (1 mg/kg t.i.w. s.c.); C, diminazene (5 mg/kg t.i.w. s.c.); D, diminazene (5 mg/kg per day s.c.). C/S, Control steroid group.

other day (q.o.d.) because of adverse reactions (severe lethargy); there was at best only slight improvement in therapeutic activity.

Aminoquinaldines. Quinapyramine was a highly effective therapy for *P. carinii* infection (Fig. 8). The median histologic score fell from 4+ in the control steroid group to 2+ in rats treated with a dose of 0.5 mg/kg per day (group A) and to 0 in animals treated with a dose of 4 mg/kg per day (group C). Dose-related effects were also noted in the quantitative studies. There was a 10-fold decline in *P. carinii* cyst counts from 2.91×10^8 per lung in the control steroid rats to 2.62×10^7 per lung in group A and almost a 1,000-fold fall to 7.21×10^5 per lung in group C rats. Nuclei counts fell 100-fold from 3.22×10^9 per lung in the control steroid group to 3.43×10^7 per lung in group A and 10,000-fold to $\leq 1.47 \times 10^5$ per lung in group C animals. All doses of quinapyramine were well tolerated.

Phenanthridimums. Isometamidium demonstrated moderate effectiveness against *P. carinii* pneumonia; this effectiveness was partially dose related (Fig. 9). Administration of isometamidium in a dose of 0.5 mg/kg per day resulted in a median histologic score of 3+ (group A), whereas doses of 3 mg/kg per day (group B) and 3 mg/kg t.i.w. (group C) resulted in scores of 1+. Median cyst counts fell from 4.38×10^8 per lung in control steroid rats to 1.13×10^8 per lung in group A, 4.33×10^7 per lung in group B, and 1.94×10^7 per lung in group C rats. Isometamidium was poorly tolerated by the animals. The drug caused severe local discomfort when injected s.c., but this route was preferred over the i.m. route, which caused tissue necrosis, or the i.p. route, which caused systemic toxicity (lethargy).

Ethidium was less active as an anti-*P. carinii* drug than was isometamidium (Fig. 10). Rats administered ethidium in doses of 0.5 mg/kg t.i.w. (group A) or 3 mg/kg t.i.w. (group

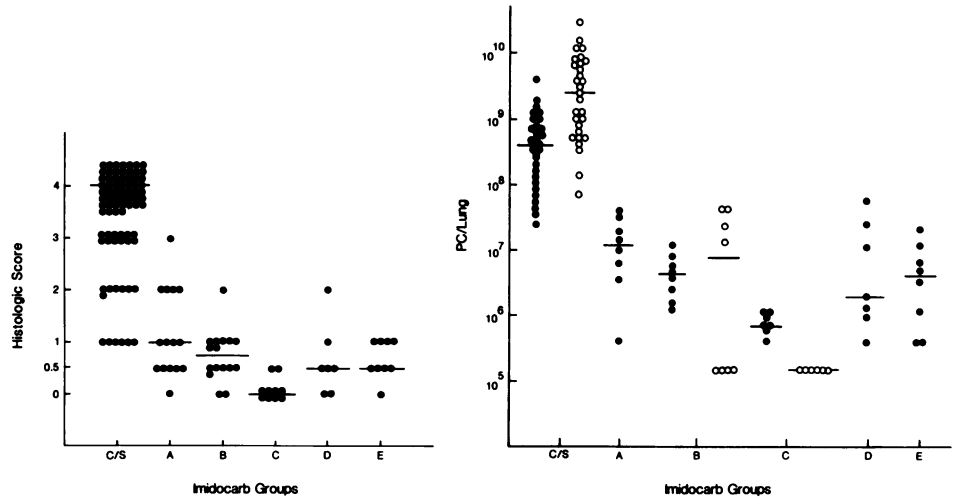

FIG. 6. Treatment of *P. carinii* infection with imidocarb as assessed by histologic score (left) and quantitation of *P. carinii* cysts (●) and nuclei (○) (right). The following imidocarb dose regimens were used: A, 2.5 mg/kg per day s.c.; B, 5 mg/kg per day s.c.; C, 10 mg/kg per day s.c.; D, 15 → 12 mg/kg per day s.c.; E, 25 → 15 mg/kg per day s.c. C/S, Control steroid group.

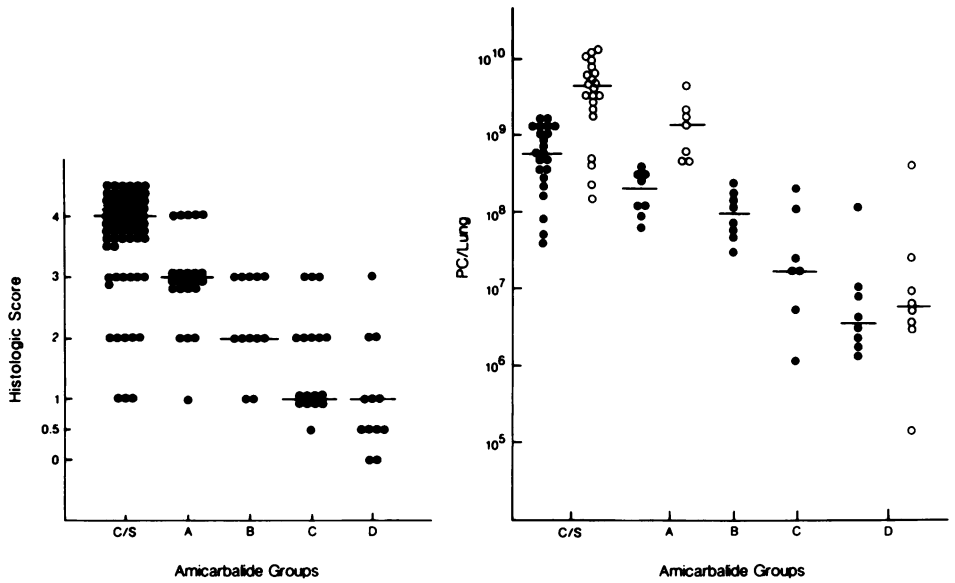

FIG. 7. Treatment of *P. carinii* infection with amicarbalide as assessed by histologic score (left) and quantitation of *P. carinii* cysts (●) and nuclei (○) (right). The following amicarbalide dose regimens were used: A, 1.5 mg/kg per day s.c.; B, 2.5 mg/kg per day s.c. → 2.5 mg/kg q.o.d. s.c.; C, 2.5 mg/kg per day s.c.; D, 10 mg/kg per day s.c. → 5 mg/kg q.o.d. s.c. C/S, Control steroid group.

B) exhibited median histologic scores and cyst counts which were very similar to those of the control steroid group animals. These doses of ethidium were given i.p., because s.c. injections of the drug caused severe local reactions. Group C rats were administered ethidium in a dose of 3 mg/kg per day s.c., which was switched to 3 mg/kg per day i.m. and then to 3 mg/kg t.i.w. i.m. because of continued poor tolerance. These animals had a median histologic score of 2+ but organism counts which were similar to those found with the other dose regimens.

Guanylhydrazones. MGBG administered in doses of 25 mg/kg t.i.w. i.p. (group A), 25 mg/kg per day s.c. (then switched to i.p.) (group B), or 50 mg/kg t.i.w. i.p. (group C) was an ineffective therapy for *P. carinii* infection as judged by histologic or quantitative criteria (Fig. 11). Although administration of MGBG in a dose of 75 mg/kg per day s.c. had to be reduced to 50 mg/kg per day i.m. because of poor tolerance (group D), this dose schedule appeared to show some anti-*P. carinii* activity. The median histologic score fell from 4+ in the control steroid group to 2+ in group D rats, and cyst and nuclei counts declined from 6.50×10^8 per lung and 4.47×10^9 per lung to 9.98×10^7 per lung and 4.50×10^8 per lung, respectively. Adverse reactions to MGBG were similar to those with ethidium.

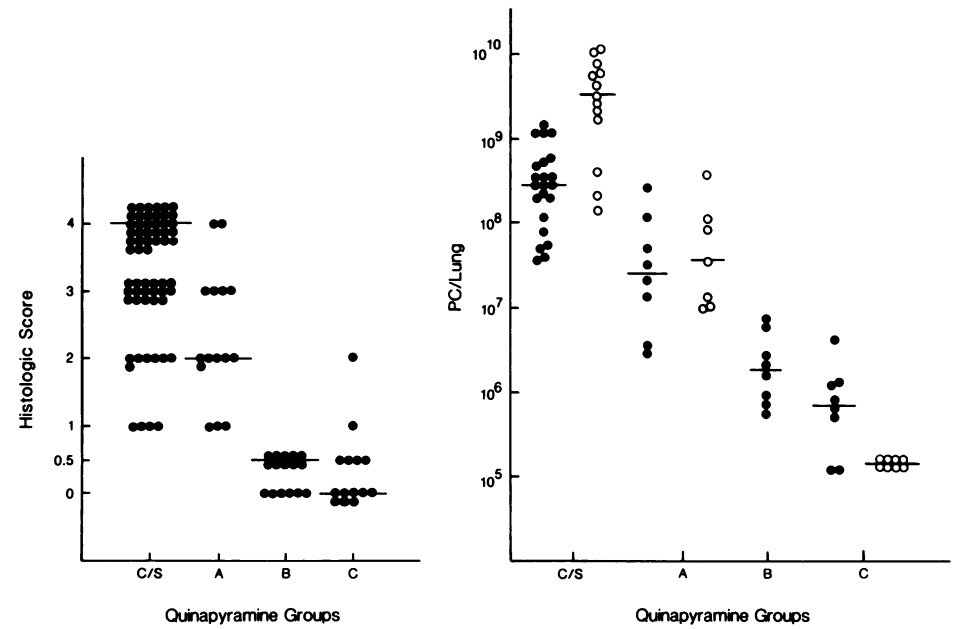

FIG. 8. Treatment of *P. carinii* infection with quinapyramine as assessed by histologic score (left) and quantitation of *P. carinii* cysts (●) and nuclei (○) (right). The following quinapyramine dose regimens were used: A, 0.5 mg/kg per day s.c.; B, 2 mg/kg per day s.c.; C, 4 mg/kg per day s.c. C/S, Control steroid group.

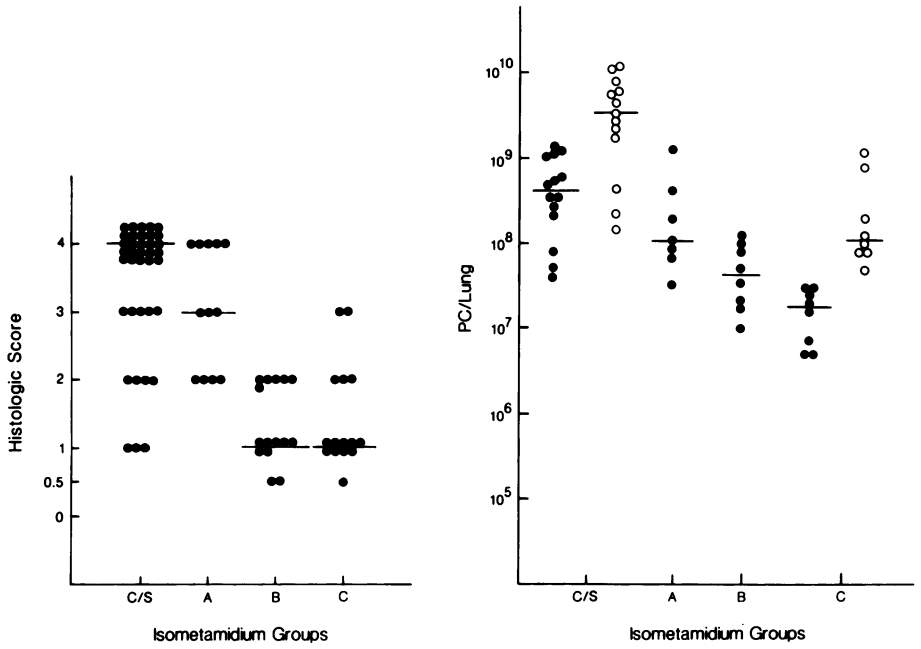

FIG. 9. Treatment of *P. carinii* infection with isometamidium as assessed by histologic score (left) and quantitation of *P. carinii* cysts (●) and nuclei (○) (right). The following isometamidium dose regimens were used: A, 0.5 mg/kg per day s.c. → 0.5 mg/kg q.o.d. s.c.; B, 3 mg/kg per day s.c.; C, 3 mg/kg t.i.w. i.p. → 3 mg/kg t.i.w. s.c. C/S, Control steroid group.

Other drugs. The following compounds were administered p.o. by gavage or by the i.m. route: quinine, 25 mg/kg per day p.o.; quinidine, 25 mg/kg per day p.o.; quinacrine, 6 mg/kg per day p.o.; chlorpromazine, 20 mg/kg per day p.o. reduced to 10 mg/kg per day p.o. because of drowsiness; spiramycin, 200 mg/kg per day p.o.; Pentostam, 10 mg/kg per day i.m.; Astiban, 10 mg/kg per day i.m.; dehydroemetine, 1 mg/kg per day i.m.; ampicillin, 150 mg/kg per day i.m.; gentamicin, 10 mg/kg per day i.m.; chloramphenicol, 20 mg/kg per day i.m.; spectinomycin, 100 mg/kg per day i.m. All drugs were ineffective therapies for *P. carinii* in the rats.

DISCUSSION

Pentamidine has served as an important standard for the development of new anti-*P. carinii* drugs. Previous reports

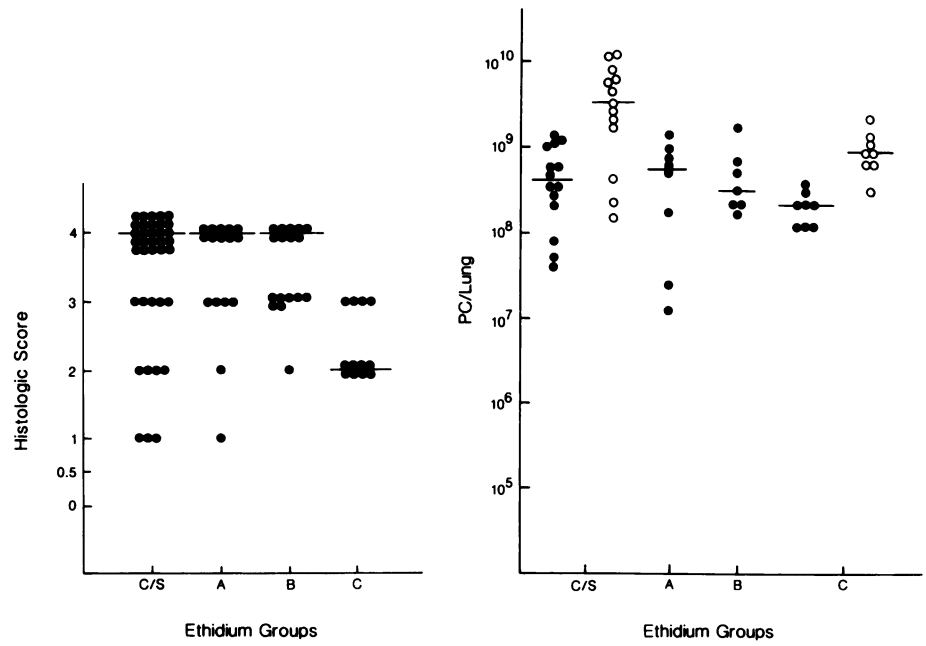

FIG. 10. Treatment of *P. carinii* infection with ethidium as assessed by histologic score (left) and quantitation of *P. carinii* cysts (●) and nuclei (○) (right). The following ethidium dose regimens were used: A, 0.5 mg/kg t.i.w. i.p.; B, 3 mg/kg t.i.w. i.p.; C, 3 mg/kg per day s.c. → 3 mg/kg per day i.m. → 3 mg/kg q.o.d. i.m. C/S, Control steroid group.

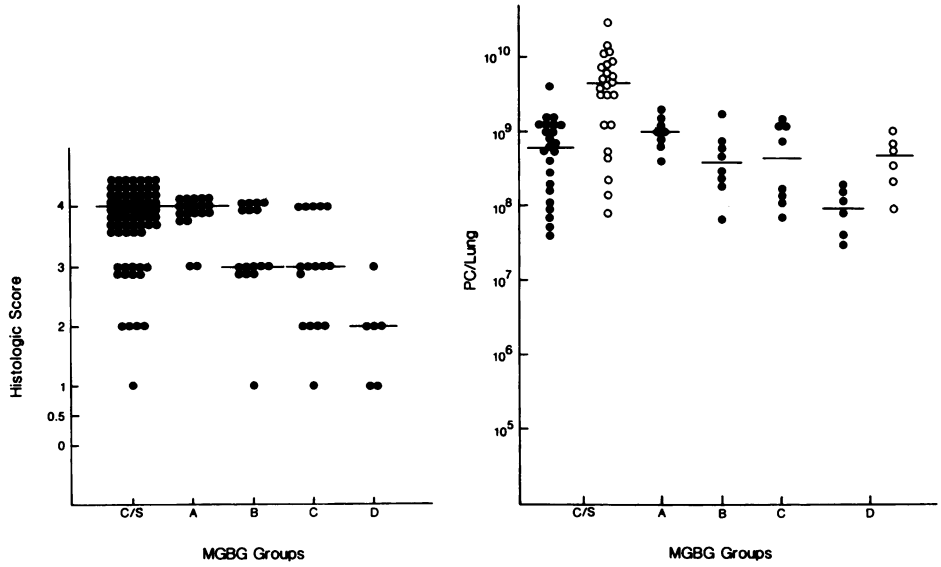

FIG. 11. Treatment of *P. carinii* infection with MGBG as assessed by histologic score (left) and quantitation of *P. carinii* cysts (●) and nuclei (○) (right). The following MGBG dose regimens were used: A, 25 mg/kg t.i.w. i.p.; B, 25 mg/kg per day s.c. → 25 mg/kg per day i.p.; C, 50 mg/kg t.i.w. i.p.; D, 75 mg/kg per day s.c. → 50 mg/kg per day s.c. → 50 mg/kg per day i.m. C/S, Control steroid group.

have demonstrated that pentamidine is moderately active in the treatment of experimental *P. carinii* infection in rats (11, 15, 23). The present study has confirmed these findings, as judged by both histology and quantitation of *P. carinii* cysts and nuclei in lung homogenates. Pentamidine causes considerable toxicity at injection sites, and this has been an important contributing factor in limiting the dose of the drug. Intravenous administration of pentamidine, which has become increasingly popular for humans, is another alternative but was not investigated in this study.

Diminazene is a diamidine which has been extensively used in the clinical treatment of veterinary African trypanosomiasis (30, 44). As with pentamidine, diminazene has also shown activity against other protozoa (e.g., *Babesia* species). This report has shown that diminazene was as effective as pentamidine in the therapy for *P. carinii* infection but was better tolerated at the injection sites by the rats. Studies such as this, which directly compare one drug with another, are complex, and the results can be influenced by experimental design as well as by drug dose, route of administration, metabolism, and pharmacokinetics. *P. carinii* pneumonia in the rat is a chronic disease, and in our protocol, treatment is begun after the infection has reached moderate intensity and is continued for 3 weeks. By contrast, experimental trypanosomiasis is usually an acute disease and therapy is given only for a few days (31).

Both pentamidine and diminazene exhibited some prophylactic anti-*P. carinii* activity, although neither drug was completely effective in preventing the development of the disease. The activity of diminazene was related to the dose and frequency of administration. Diminazene has a shorter half-life than pentamidine and has had much greater use in the therapy than in the prophylaxis of trypanosomiasis (44).

Since drugs which are active against *P. carinii* in the rat model are usually active against *P. carinii* infection in humans, diminazene may have clinical application. Diminazene appears to be one of the few cationic trypanocidal agents other than pentamidine or hydroxystilbamidine to have been administered to humans; the drug has had limited use in the treatment of African trypanosomiasis and babesiosis (2, 20, 38) but has never undergone formal evaluation in a controlled clinical setting. Caution is advised in any contemplated human study of diminazene, given the known general toxic properties of the diamidines and the heightened susceptibility of AIDS patients to adverse drug reactions. Data obtained with diminazene in the present study suggest that other diamidine compounds may also have anti-*P. carinii* activity; however, these drugs are old and may no longer be manufactured. For example, we were unable to obtain sufficient quantities of hydroxystilbamidine for testing in rats.

DFMO, an inhibitor of polyamine biosynthesis which is structurally unrelated to the diamidines, has been shown to have antitrypanosomal properties both in vivo and in vitro (3, 28, 29). The drug has been successful in the treatment of *P. carinii* infection in humans and has exhibited activity against *P. carinii* infection in rat tissue culture (8, 28). In the rat model of *P. carinii* pneumonia, DFMO was ineffective when administered as a 2% oral solution (16) but did demonstrate activity against the organism when given as a 3% solution (A. B. Clarkson, D. E. Williams, and C. Rosenberg, submitted for publication). The present study has shown that DFMO in doses similar to those reported above lacked therapeutic efficacy when used alone or in combination with the diamidines. The reasons for these conflicting results are unclear but may involve factors (e.g., experimental design, drug pharmacokinetics) discussed above. DFMO was also poorly tolerated by our rats, thus illustrating the problem of drug toxicity encountered in this immunosuppressed animal population.

The carbanilides, imidocarb and amicarbalide, resemble the diamidines in structure but differ in bridging groups. These drugs have been used clinically in veterinary medicine to treat *Babesia* and *Anaplasma* infections and experimentally to treat *Trypanosoma brucei brucei* infection in mice (21, 31, 37). In this study, imidocarb was highly active and amicarbalide was moderately active against *P. carinii* infection in rats. It has been suggested that drugs closely related to the carbanilides (e.g., phthalanilides) be explored for

antitrypanosomal activity (31); such an idea might also be applied to *P. carinii*.

Quinapyramine, an aminoquinaldine derivative discovered in 1949, has received extensive clinical use in the treatment and prophylaxis of African trypanosomiasis in animals (6, 30, 44). The drug has been prepared in two forms, the methylsulfate and chloride, which have different pharmacologic properties. As with other cationic trypanocidal drugs, quinapyramine has considerable toxicity; the most prominent adverse effect is a curarelike reaction. The quinapyramine preparation obtained for use in this study had a shelf life which exceeded the recommended expiration date. Nevertheless, the drug was a highly effective therapy for *P. carinii* infection, with clear-cut activity seen with each dose used. A variety of other aminoquinaldine compounds have been synthesized (44).

The phenanthridiniums have been a major class of cationic veterinary antitrypanosomal agents for many years (30, 44). Early drugs, phenidium and dimidium, were replaced by the compounds which had specific structural characteristics: ethidium, which had an ethyl group substituted for the methyl group on the quaternary nitrogen atom; prothidium, which had the pyrimidyl group of quinapyramine; and isometamidium, which had the aminobenzamidine moiety of diminazene. A variety of other agents have also been investigated (5, 45). In this study, isometamidium was a moderately effective therapy for *P. carinii* pneumonia, but ethidium showed only slight activity. Both compounds caused severe local toxicity, which is typical of drugs for this class and which may have limited their effectiveness in the rat model.

MGBG, a guanylhydrazone similar to pentamidine in having two terminal amidine groups, has antineoplastic and antitrypanosomal properties. Recently, a variety of analogs of MGBG have been synthesized and investigated in an attempt to learn more about structure-antitrypanosomal activity relationships (40, 41). The present study has shown that MGBG has slight anti-*P. carinii* properties but causes significant local toxicity. Perhaps exploration of other guanylhydrazone analogs would be more fruitful.

Taken together, the data obtained in the present study suggest that anti-*P. carinii* activity is a common property of cationic trypanocidal compounds. One important implication of this finding is that it should help focus efforts to develop anti-*P. carinii* drugs. Such a situation has occurred with inhibitors of folic acid synthesis, in which a variety of new and old drugs have recently been explored in the therapy for *P. carinii* infection (1, 17–19, 25, 33, 42). As can be seen in the present study, as well as in previous reports (11, 15, 16), empiric screening of a broad array of antiparasitic and other antimicrobial agents has been an inefficient procedure for discovering useful compounds.

Among the cationic agents, it should now be possible to begin to study structure-activity relationships. The wealth of background information obtained with trypanosomes should be helpful in targeting compounds and in data analysis. It will probably be necessary to synthesize drugs for study, given the problems we have encountered in obtaining some of these entities. The rat model will continue to play a prominent role in drug evaluation, but with recent improvement in in vitro techniques (1, 4, 8), it is likely that other systems will also be used.

It is hoped that investigation of these cationic drugs will lead to improved chemotherapy for *P. carinii* infection and aid in studies of the basic biology of the organism. At present, only a few metabolic studies of *P. carinii* have been performed (27, 34–36); however, with the marked increase in research interest in the organism, it is likely that progress in this area will be made. The availability of well-defined compounds with known effects on *P. carinii* should be helpful in studying the biochemical pathways of the organism and elucidating mechanisms of drug action.

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