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# Pentamidine Aerosol in Prophylaxis and Treatment of Murine *Pneumocystis carinii* Pneumonia

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**The efficacy and tolerance of pentamidine aerosol were evaluated in the prophylaxis and therapy of murine *Pneumocystis carinii* pneumonia. *P. carinii* pneumonia was induced in rats by corticosteroid immunosuppression. Pentamidine was administered three times weekly via a Bird micronebulizer. The actual amount of pentamidine inhaled was estimated by monitoring the ventilation of the rats during the aerosol administration. Pentamidine levels in blood, lung, liver and kidney samples were determined by high-pressure liquid chromatography after completion of the treatment. Efficacy was evaluated by examination of lung imprints. In the prophylactic treatment, 4.8- and 8.6-mg/kg doses of aerosolized pentamidine administered three times weekly for 7 weeks were effective in preventing *P. carinii* pneumonia in 80 and 100% of the rats, respectively. In the therapeutic studies, a 14.6-mg/kg dose of aerosolized pentamidine administered three times weekly for 3 weeks was effective both in curing the pneumonia and in clearing *P. carinii* cysts in 70% of the rats. In the remaining animals, although the pneumonia was cured, the cysts persisted. A dose-dependent effect of the drug was demonstrated in both prophylactic and therapeutic treatments. High lung/kidney and lung/liver ratios of pentamidine levels were demonstrated and were associated with good clinical, biological, and histologic tolerance.**

More than 10,000 cases of *Pneumocystis carinii* pneumonia have been reported in the United States to the Centers for Disease Control since the emergence of the acquired immunodeficiency syndrome epidemic (5). *P. carinii* infection is confined to the lungs. Trimethoprim-sulfamethoxazole (TMP-SMZ) and pentamidine are the two drugs which are routinely used in treating human *P. carinii* pneumonia. However, the parenteral administration of pentamidine is hampered by frequent local intolerance and major systemic side effects due to its high level of extrapulmonary deposition. TMP-SMZ side effects are also frequent, especially in acquired immunodeficiency syndrome patients. Since preliminary data on aerosolized pentamidine pharmacokinetics were available (E. M. Bernard, H. P. Donelly, H. P. Koo, and D. Amstrong, Program Abstr. 25th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 552, 1985), we tested the feasibility, tolerance, and efficacy of pentamidine aerosol in the prophylaxis and therapeutic treatment of murine *P. carinii* pneumonia.

## MATERIALS AND METHODS

**Animal model of *P. carinii* pneumonia.** Male Sprague-Dawley rats (Charles River Breeding Laboratories, St. Aubin, France), weighing 200 to 250 g, were housed in groups of five in conventional cages with standard rat chow (no. A03; Usine Alimentation Rationelle, Villemoisson, France) and water ad libitum. Rats were weighed weekly.

Rats were immunosuppressed by subcutaneous injections of 25 mg of cortisone acetate (Hydrocortisone; Hoechst-Roussel, Paris, France) twice weekly, until death or sacrifice. Doxycycline (Vibramycine; Pfizer Inc., Paris, France) was subcutaneously injected at a dose of 10 mg twice weekly to prevent bacterial superinfection. This model has been proved to induce *P. carinii* pneumonia after 5 to 6 weeks of treatment in 90 to 100% of rats (10, 11).

**Aerosol exposure system.** Pentamidine aerosol was delivered three times weekly for 5 to 10 min individually to each rat with a micronebulizer (Bird Corporation, Palm Springs, Calif.) driven by compressed air at 7 to 7.5 lb/in$^2$.

Under these conditions, 25% of the aerosolized particles have a diameter up to 5 μm, which is required for alveolar deposition (14). The nebulizer reservoir was filled with at least 4 ml of pentamidine (pentamidine mesylate, Lomidine; Specia, Paris, France). The rat was introduced into a plethysmograph (Battelle Centre for Toxicology and Biosciences, Geneva, Switzerland), and its emerged head was connected to the Bird nebulizer. The ventilation was measured during the aerosol delivery by the method of Coggins et al. (6). The amount of pentamidine aerosolized was determined by weighing the reservoir of the nebulizer before and after delivery. The estimated dose (in milligrams) of pentamidine inhaled ($I$) was calculated by using the following formula: $I = C \times D \times V_E$, where $C$ is the concentration of pentamidine in the reservoir (in milligrams per milliter), $D$ is the duration (in minutes) of aerosol administered, and $V_E$ is the ventilation (in milliliters per minute). The dose was expressed per kilogram of rat body weight.

**Experimental design. (i) Control group.** Seven rats were not immunosuppressed but were submitted to pentamidine aerosol to evaluate pentamidine pharmacokinetics and tolerance. The animals were divided into two groups equivalent to the two pentamidine aerosol schedules of the immunosuppressed rats (see prophylactic and therapeutic treatments). In group 1, two rats were given 3.8 mg and two rats were given 6.6 mg of aerosolized pentamidine per kg three times weekly for 7 weeks. In group 2, three rats were given 8.7 mg of aerosolized pentamidine per kg three times weekly for 3 weeks.

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**(ii) Prophylactic-treatment protocol.** Twenty-seven rats were randomly assigned to three groups from the initiation of immunosuppression. In group 3, 12 rats received no anti-*P. carinii* drug. Three of them could not be evaluated because of premature death. In group 4, five rats were treated by TMP-SMZ injected subcutaneously twice weekly (TMP, 40 mg/kg; SMZ, 200 mg/kg). In group 5, 10 rats were treated with pentamidine aerosol three times weekly. The mean inhaled dose of each aerosol was 4.8 mg/kg in five rats and 8.6 mg/kg in the other five rats. All of the rats in these groups were sacrificed after 7 weeks.

**(iii) Therapeutic-treatment protocol.** TMP-SMZ injections or pentamidine aerosols were initiated after either 5 or 6 weeks of immunosuppression. Thirty-three rats were randomly assigned to three groups. In group 6, 12 rats received no anti-*P. carinii* drug. In group 7, four rats were treated with subcutaneous TMP-SMZ twice weekly (TMP, 40 mg/kg; SMZ, 200 mg/kg) after 5 weeks of corticosteroid administration. In group 8, 17 rats were treated with pentamidine three times weekly and divided into the two following subgroups. In subgroup 8a, seven rats were administered pentamidine aerosol at the mean inhaled dose of 4.7 mg/kg and 6 mg/kg in three and four rats, respectively, after 6 weeks of immunosuppression. In subgroup 8b, 10 rats were administered pentamidine aerosol at the mean inhaled dose of 14.6 mg/kg after 5 weeks of immunosuppression. After 3 weeks of treatment, the surviving rats were sacrificed and studied as described above.

**Blood and tissue sampling.** At the end of the course and 24 h after the last administration of aerosol, the surviving rats were anesthetized with pentobarbital (Penthotal; Abbot, Orsay, France). They were exsanguinated via the abdominal aorta with a VACUTAINER (Becton Dickinson, Meylan, France). The lungs, kidneys, and livers were removed, examined macroscopically, and weighed.

Lung imprints and Formalin-fixed histologic sections of several lobes of both lungs were stained with silver methenamine (Gomori-Grocott) and coded. Histologic sections of lungs, kidneys, and livers were prepared and stained with hemateine-phloxine-safran after Formalin fixation and coded. Immediately after histologic sampling, the organs were homogenized in 1.5 ml of phosphate-buffered saline, frozen at −70°C, and kept for measurement of pentamidine concentration.

**Evaluation of tolerance and efficacy of aerosolized pentamidine.** Tolerance was evaluated by biochemical determination of the levels of transaminases (ASAT, ALAT), amylase, and creatinine in plasma and by histologic examination of lungs, kidneys, and liver.

The efficacy of pentamidine aerosol was assessed through coded lung imprint examination (160-fold magnification) by two pathologists. According to the degree of *P. carinii* infection, three groups were distinguished: (i) none, no cysts; (ii) rare, less than 10 scattered cysts per field; and (iii) numerous, clusters of many cysts per field. A discordance between the two pathologists occurred three times, and the more-infected group was recorded. Histologic sections were examined for characteristic *P. carinii* alveolitis.

**Measurement of pentamidine concentration.** Samples of plasma, lung, kidney, and liver were taken 24 h after the last aerosol treatment. The pentamidine concentration was determined by high-pressure liquid chromatography and fluorometry. Briefly, a volume of the homogenate equivalent to 500 mg of tissue was extracted under alkaline conditions with organic solvents in the presence of hexamidine as an internal standard (15). It was concentrated in an acid solution and reacted with glyoxal and benzaldehyde. Derivatives of pentamidine and hexamidine were then extracted in an ethyl acetate-isopropanol (9:1) mixture. The organic layer was concentrated, the sample was reconstituted by adding acetonitrile, and separation was effected by reversed-phase liquid chromatography. A $C_{18}$ column with a 5-μm particle size was used (Regis Chemical, Morton Grove, Ill.). The mobile phase was prepared by premixing and filtering 100 ml of distilled water and 400 ml of acetonitrile. The flow rate was 1 ml/min. Fluorometric detection was monitored at 450 nm with the excitation wavelength set at 400 nm. Plasma extraction was done with a C8 SPE column (J. T. Baker Chemical Co., Phillipsburg, N.J.). The eluate was then derived as described for tissues. The intra-run and run-to-run reproducibility was ±7.1% for a concentration of 0.5 μg/g of tissue and ±6.4 for a concentration of 0.3 μg/ml of plasma. The detection limit in plasma was 0.05 μg/ml.

## RESULTS

**Prophylactic-treatment protocol.** The nine nontreated rats were heavily infected with *P. carinii* cysts and showed the characteristic pneumonia. TMP-SMZ was always efficient in preventing the infection (5/5). Pentamidine aerosols appeared to be as efficient as TMP-SMZ when delivered in a 8.6-mg/kg dose. At a dose of 4.8 mg/kg, they were effective in four of the five rats; the only infected rat exhibited occasional *P. carinii* cysts without the characteristic pneumonia (Table 1). Mean pentamidine concentrations are shown in Table 2. The concentration of pentamidine was dose dependent with high levels in the lung compared to the kidney and liver. The pentamidine kidney/lung and liver/lung ratios were approximately 1/10 and less than 1/100, respectively. No pentamidine could be detected in the plasma by this high-pressure liquid chromatography assay.

**Therapeutic-treatment protocol.** Most of the nontreated rats (10/12) died before the end of the 9-week corticosteroid course. Nine of those which could be evaluated exhibited *P. carinii* pneumonia. TMP-SMZ was active in three of the four rats; the fourth rat exhibited very few *P. carinii* cysts (Table 3).

As with the prophylactic schedule, pentamidine aerosol had a dose-dependent effect. At the low dose (4.7 mg/kg), pentamidine aerosol was not effective in eradicating the infection. However, with the maximal dose used (14.6 mg/kg), 70% of the rats (7/10) were completely cured, while the remaining 30% (3/10) showed occasional cysts without characteristic *P. carinii* pneumonia (Table 3). Mean pentamidine concentrations are shown in Table 4. A good

TABLE 1. Efficacy of pentamidine aerosols versus TMP-SMZ in prophylactic treatment protocol

| Group[a] | Treatment and dose (mg/kg) | No. of animals with *P. carinii* cysts | | |
|---|---|---|---|---|
| | | None | Rare | Numerous |
| 3 | None | 0 | 0 | 9 |
| 4 | TMP-SMZ (s.c., 40-200)[b] | 5 | 0 | 0 |
| 5 | Pentamidine aerosol | | | |
| | 4.8 | 4 | 1 | 0 |
| | 8.6 | 5 | 0 | 0 |

[a] Rats were evaluated after 7 weeks of immunosuppression. In group 3, three rats died prematurely and were not evaluated. In group 4, five rats were injected twice weekly with TMP-SMZ. In group 5, ten rats received aerosol treatments three times weekly.

[b] s.c., Subcutaneous.

correlation between the amount of aerosolized pentamidine and the concentration in the lungs was observed. Moreover, the concentration in the lungs was lower in the rats with *P. carinii* pneumonia than in the nonimmunosuppressed rats: 8.7 mg/kg in three control rats led to a higher concentration than 14.6 mg/kg in the infected animals. The pentamidine kidney/lung and liver/lung ratios varied between 1/4.5 to 1/6 and 1/60 to 1/160, respectively. No pentamidine was detected in the plasma in this therapeutic protocol.

**Appraisal of tolerance to pentamidine aerosol.** Plasma transaminase, amylase, and creatinine levels were measured in 6 rats from the control group, in 7 rats from the prophylactic-treatment protocol, and in 11 rats from the therapeutic-treatment protocol. No significance difference appeared between the three groups. No histologic abnormality of lung, liver, and kidney could be found in the pentamidine aerosol-treated rats.

## DISCUSSION

Under the conditions of these experiments, aerosolized pentamidine appeared to be nontoxic and able to prevent and even cure *P. carinii* pneumonia in the rat. A trial of pentamidine aerosol therapy and prophylaxis in humans is justified by the need to avoid the frequent systemic side effects observed in humans and to deliver the drug rapidly to the lungs, since no pentamidine could be detected within lungs of patients given pentamidine intramuscularly or intravenously during the first 4 days of administration (2; H. Donelly, E. M. Bernard, H. E. Rothkotter, J. M. V. Gold, and D. Armstrong, 26th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 696, 1986). Moreover, permanent immunodeficiency states (e.g., acquired immunodeficiency syndrome) require effective prophylaxis of *P. carinii* pneumonia without side effects.

Aerosol treatment of bacterial and viral pneumonia has already been suggested because of the accessible route and the requirement of high levels of the antimicrobial agent in the lungs (1). One major problem of aerosol administration is the difficulty in determining the actual amount of drug reaching the alveoli, since a significant fraction of the drug is lost within the nebulizer and in the upper airways. In the present study, the ventilation monitoring was intended to partially overcome this difficulty. Furthermore, the alveolar disease itself, as we have shown, can alter the extent of drug penetration. Studies of antimicrobial aerosols are very rare, but recent works have reported successful results with this route of administration in treatment of Legionnaires disease in guinea pigs (9), as well as in the treatment of respiratory syncytial virus infection in infants (3) and of human adenovirus pneumonia with ribavirin aerosol (4). The interest in this route of administration has recently been emphasized by Debs et al. (8), who demonstrated success with pentamidine aerosol in a murine model of *P. carinii* pneumonia that was similar to ours. Immunosuppression was induced by oral corticosteroids, and a different technique of aerosolization was applied. Pentamidine, free or encapsulated in liposomes, was administered by aerosolization of a chamber enclosing the rats. Aerosol treatments were compared with intravenously administered pentamidine in curative courses after 5 or 6 weeks of immunosuppression.

In our model, TMP-SMZ injected twice weekly prevented *P. carinii* pneumonia in all rats as already shown (13), although other researchers usually administer it orally every day. Aerosols of pentamidine appear to be very effective at 8.6 mg/kg three times weekly in the prevention of *P. carinii* pneumonia in this murine model. The efficacy of these aerosols is comparable with that of TMP-SMZ, and it clearly correlates with the high levels of pentamidine obtained in lungs. Comparison of our results with those of Western et al. (16) using intramuscular pentamidine (4 mg/kg daily), a dose which did not prevent *P. carinii* pneumonia, supports the potential value of aerosol therapy for prophylaxis. The lack of biological abnormalities was probably linked with the high lung/kidney and lung/liver pentamidine concentration ratios. Nevertheless, pentamidine eliminated via the kidney was

TABLE 2. Levels of pentamidine in rats treated with pentamidine aerosols during prophylactic-treatment protocol

| Group[a] | Treatment and dose of pentamidine (mg/kg) | No. of animals | Mean pentamidine concn (μg/g) ± SEM in: | | |
|---|---|---|---|---|---|
| | | | Lung | Liver | Kidney |
| 1 | Control (nonimmunosuppressed) | | | | |
| | 3.8 | 2 | 172 ± 23 | 0.61 ± 0.38 | 3 ± 1 |
| | 6.6 | 2 | 227 ± 49 | 0.34 ± 0.03 | 9 ± 1 |
| 5[b] | Immunosuppressed | | | | |
| | 4.8 | 4 | 147 ± 24 | 0.4 ± 0.1 | 14 ± 2 |
| | 8.6 | 5 | 210 ± 15 | 1.6 ± 0.3 | 20 ± 2 |

[a] All rats received the pentamidine aerosol three times weekly. Animals were sacrificed 24 h after the last dose of a 7-week course of pentamidine aerosol.

[b] See Table 1, footnote *a*.

TABLE 3. Efficacy of pentamidine aerosols versus TMP-SMZ after 3 weeks of therapeutic-treatment protocol

| Group[a] | Treatment and dose (mg/kg) | No. of animals with *P. carinii* cysts | | |
|---|---|---|---|---|
| | | None | Rare | Numerous |
| 6 | None | 0 | 0 | 9 |
| 7 | TMP-SMZ (s.c., 40-200)[b] | 3 | 1 | 0 |
| 8 | Pentamidine aerosol | | | |
| 8a | 4.7 | 0 | 0 | 3 |
| | 6 | 0 | 3 | 1 |
| 8b | 14.6 | 7 | 3 | 0 |

[a] In group 6, three rats died before week 7 and were not evaluated, seven rats died between the week 7 and 8 but could be examined, and the last two rats were sacrificed at week 9. In group 7, four rats received TMP-SMZ twice weekly after 5 weeks of immunosuppression. In group 8a, rats were treated with three times weekly pentamidine aerosol after 6 weeks of immunosuppression; three received 4.7 mg/kg and four received 6 mg/kg per aerosol. In group 8b, 10 rats received pentamidine aerosol three times weekly (14.6 mg/kg per aerosol) after 5 weeks of immunosuppression.

[b] s.c., Subcutaneous.

TABLE 4. Levels of pentamidine in tissues of rats treated with pentamidine aerosols (control and therapeutic-treatment protocol)

| Group | Pentamidine (mg/kg) | No. of animals | Mean pentamidine concn (μg/g) ± SEM in: | | |
|---|---|---|---|---|---|
| | | | Lung | Liver | Kidney |
| 2[a] | Control (nonimmunosuppressed), 8.7 | 3 | 210 ± 20 | 0.58 ± 0.06 | 15 ± 4 |
| 8[b] | Immunosuppressed | | | | |
| 8a | 4.7 | 3 | 18 ± 8 | 0.3 ± 0.1 | 4 ± 1 |
| 8b | 6 | 4 | 42 ± 9 | 0.26 ± 0.03 | 7 ± 1 |
| 8b | 14.6 | 8 | 128 ± 17 | 1.4 ± 0.2 | 26 ± 5 |

[a] Three nonimmunosuppressed rats were treated with pentamidine aerosol three times weekly for 3 weeks.

[b] See Table 3, footnote *a*. In subgroup 8b, two rats could not be analyzed.

detected in this organ, and dose adjustment could be necessary in courses of longer duration.

The results of aerosolized-pentamidine treatment of established infection appear promising. The dose required is much higher than that needed for prophylactic purposes and could be related to the pathologic lesions of the *P. carinii* pneumonia. As the severity of alveolitis increased with the duration of the immunosuppression, the protocol of curative treatment of rats of subgroup 8b was modified. Pentamidine was introduced with a higher dose after 5 instead of 6 weeks of corticosteroid treatment. At a dose of 14.6 mg/kg, given three times weekly, the efficacy was excellent in 70% of the rats and the remaining rats were poorly infected without histologic features of *P. carinii* pneumonia. The necessity of earlier administration of anti-*P. carinii* drugs was demonstrated with conventional delivery (12) and with pentamidine aerosol by Debs et al. (8). The extrapulmonary depositions, i.e., in liver and kidneys, was low as observed by Debs et al., who compared them to deposition after intravenous injection. However, it was proportionately higher than in prophylaxis, a finding which could be explained by an increasing pulmonary blood output in the infected lungs. This emphasizes the need for biochemical monitoring for potential toxicity, although no abnormality was observed in this series. Both in prophylactic and in therapeutic courses, the levels of pentamidine in lung were much higher than the minimal concentration determined to be effective in vitro (0.1 μg/ml) (7).

Pentamidine was not detectable in the plasma 24 h after the last administration of aerosol. This is consistent with the pharmacological data of intravenously injected pentamidine, which is characterized by a very short serum half-life (2 min in rats) and a long half-life of urinary excretion (several days) (2).

More studies on the pharmacology of pentamidine delivered by the aerosol route are warranted before human trials can be conducted. New, more efficient ultrasonic-type nebulizers could facilitate the aerosol delivery mainly by generating smaller particles with improved alveolar penetration.

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## LITERATURE CITED

1. **Berendt, R. F., G. G. Long, and J. S. Walker.** 1975. Treatment of respiratory *Klebsiella pneumoniae* infection in mice with aerosols of kanamycin. Antimicrob. Agents Chemother. **8**:585–590.
2. **Bernard, E. M., H. J. Donnelly, M. P. Maher, and D. Armstrong.** 1985. Use of a new bioassay to study pentamidine pharmacokinetics. J. Infect. Dis. **152**:750–754.
3. **Breese Hall, C., J. T. McBride, E. E. Walsh, D. M. Bell, C. L. Gala, S. Hildreth, L. G. Ten Eyck, and W. J. Hall.** 1983. Aerosolized ribavirin treatment of infants with respiratory syncytial viral infection. A randomized double-blind study. N. Engl. J. Med. **308**:1443–1447.
4. **Buchdahl, R. M., P. Taylor, and J. O. Warner.** 1985. Nebulised ribavirin for adenovirus pneumonia. Lancet **ii**:1070–1071.
5. **Centers for Disease Control.** 1986. Update. Acquired immunodeficiency syndrome—United States. Morbid. Mortal. Weekly Rep. **35**:17–21.
6. **Coggins, C. R. E., F. Duchosal, C. Musy, and R. Ventrone.** 1981. Measurement of respiratory patterns in rodents using whole body plethysmography and pneumotachograph. Lab. Anim. **15**:137–140.
7. **Cushion, M. T., D. Stanforth, 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.
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. **Fitzgeorge, R. B., A. Baskerville, and A. S. R. Featherstone.** 1986. Treatment of experimental Legionnaires' disease by aerosol administration of rifampicin, ciprofloxacin, and erythromycin. Lancet **i**:502–503.
10. **Frenkel, J. K., J. T. Good, and J. A. Schultz.** 1966. Latent pneumocystis infection in rats, relapse and chemotherapy. Lab. Invest. **15**:1559–1577.
11. **Hugues, T. H., P. McNabb, T. D. Makres, and S. Feldran.** 1974. Efficacy of trimethoprim and sulfamethoxazole in the prevention and treatment of *Pneumocystis carinii* pneumonitis. Antimicrob. Agents Chemother. **5**:289–293.
12. **Hugues, W. T., and B. L. Smith.** 1984. Efficacy of diaminodiphenylsulfone and other drugs in murine *Pneumocystis carinii* pneumonitis. Antimicrob. Agents Chemother. **26**:436–440.
13. **Kernbaum, S., J. Masliah, L. G. Alcindor, C. Bouton, and D. Christol.** 1983. Phospholipase activities of bronchoalveolar lavage fluid in rat *Pneumocystis carinii* pneumonia. Br. J. Exp. Pathol. **64**:75–79.
14. **Newman, S. P.** 1985. Aerosol deposition considerations in inhalation therapy. Chest **88**(Suppl.):1525S–1605S.
15. **Waalkes, T. P., and V. T. de Vita.** 1970. The determination of pentamidine (4,4′ diamino phenoxy pentane) in plasma, urine and tissues. J. Lab. Clin. Med. **75**:871–878.
16. **Western, K. A., L. Norman, and A. F. Kaufmann.** 1975. Failure of pentamidine isothionate to provide chemoprophylaxis against *Pneumocystis carinii* infection in rats. J. Infect. Dis. **131**: 273–276.