Lobar Pentamidine Levels and *Pneumocystis carinii* Pneumonia Following Aerosolized Pentamidine

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Recent studies have suggested that failure of pentamidine prophylaxis against *Pneumocystis carinii* pneumonia (PCP) may be due to reduced deposition of pentamidine in the upper lobes. In this study, we performed bronchoalveolar lavage from the apical segment of the upper lobe and the middle lobe in 51 HIV-positive patients, all of whom were receiving prophylaxis with aerosolized pentamidine, who had presented with acute respiratory symptoms. Lavage fluid from each lobe was assayed for pentamidine using high-performance liquid chromatography (HPLC). The number of clusters of *P carinii* were counted after staining with a Wright-Giemsa stain. The patients were subclassified as PCP-positive (32 patients) and PCP-negative (19 patients) on the basis of the presence/absence of *P carinii* clusters in their BAL fluid. The concentration of pentamidine in the upper lobe compared with the middle lobe was no different (using paired Student's t tests) for either PCP-positive patients or PCP-negative patients. In comparing the positive with the negative subjects, using unpaired Student's t tests, there was no difference in the concentration of pentamidine in the upper lobe or the middle lobe. For PCP-positive patients, the numbers of *P carinii* clusters were on average higher in the upper lobes (mean ± SD: upper = 14.9 ± 16.6, middle 7.5 ± 10.8, p = 0.013, paired Student's t test), but there was no correlation between lobar *P carinii* cluster counts and pentamidine levels. We conclude that the absence of a relationship between cluster count and pentamidine level, the similarity in regional pentamidine levels between upper and middle lobes, as well as the similarity in pentamidine levels between the PCP-positive and PCP-negative groups indicate that the regional dose of pentamidine is not the determining factor as to whether aerosolized pentamidine prophylaxis will succeed or fail.

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**METHODS**

*Patient Population*

Fifty-one human immunodeficiency virus (HIV)-positive patients were studied. The study had been approved by the hospital ethics committee and informed written consent obtained from each patient. All were receiving primary or secondary prophylaxis with AP at the University of Cincinnati Medical Center. Pentamidine 300 mg was delivered monthly using a nebulizer (Respigard II, Marquest, Englewood, Colo). Patients inhaled the drug in a sitting position. Entry requirements consisted of a history of AP prophylaxis and clinical presentation with acute respiratory symptoms. Patients were recruited consecutively over a 4-month period. None of the patients had received intravenous pentamidine for at least 6 months prior to the study. The patients were subdivided into those who had detectable *P carinii* clusters in bronchoalveolar lavage (BAL) fluid (32 PCP-positive subjects) and those in whom *P carinii*...
was not detectable (19 PCP-negative subjects).

Bronchoalveolar Lavage

The BAL was performed using a bronchoscope that was wedged in the segment to be lavaged, as part of the patient’s diagnostic evaluation. In every subject, BAL was performed in the right middle lobe and the apical segment of the right upper lobe, except for those patients who had predominantly left-sided disease, in whom BAL was performed in the lingula and apical-posterior segment. The PCP-positive and PCP-negative subjects had a similar proportion of left-sided BAL (4/32 and 3/19, p = NS, \( \chi^2 \)).

During the lavage procedure, the suction port was clamped and 120 ml of normal saline solution were instilled in aliquots of 60 ml into the middle lobe/lingula with each aliquot immediately aspirated via a large hand-held syringe. This procedure was repeated in the upper lobe.

Pentamidine Analysis

Aliquots of 5 ml of raw BAL fluid were obtained from each of the two lobes as described above. The supernatant and sediment were analyzed for pentamidine by high-performance liquid chromatography (HPLC) using a previously described protocol. Each sample was centrifuged at 1,000 g for 10 min. The supernatant was decanted and spiked with a hexamidine standard. Then, the supernatant was passed through a cartridge (C-8 Bond Elut) and washed with water, 50 percent methanol, and 100 percent methanol alcohol. Finally, the sample was eluted with 97.5 percent methanol alcohol/0.5 percent sodium-1-heptane sulfonate/0.02 percent (10 percent) tetramethyl-ammonium chloride/0.1 percent H\(_3\)PO\(_4\) qs to 100 percent with distilled water. The eluent was concentrated under nitrogen and passed through a 5-μm column (Alttech Ultraphase Octyl) using an automated detection system (Shimadzu Scientific Instruments Inc, Princeton, NJ). The pellet of sediment was resuspended in 1 ml phosphate buffer 0.9 percent saline and hexamidine was added. Then, the solution was precipitated with 1 ml of pure acetone and centrifuged. The supernatant from this precipitation was eluted as above and analyzed. All samples were analyzed in duplicate to confirm reproducibility.

Pneumocystis Quantitation

Cyto centrifuge-prepared slides were made of additional 5-ml aliquots of the samples from the upper and middle lobe and stained with a Wright-Giemsa stain. Clusters of \( P \) carinii organisms were quantitated by counting 500 nucleated cells and noting the number of \( P \) carinii clusters found. Two slides from each lobe were made and the counts were averaged.\(^a\)

| Table 1 — Mean (± SD) Concentration of Pentamidine (ng/ml of BAL Fluid) for Upper and Middle Lobes Compared Using Paired Student’s t Tests* |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                | PCP-Positive Patients |                 |                 | PCP-Negative Patients |                 |                 | Unpaired Student’s t Test Positive vs Negative |                 |
|                                | Counts | Pentamidine, ng/ml | Total | Supe | CP | Total | Supe | CP | Total | p = NS | p = NS | p = NS |
| Upper lobe                     |         |                   |       |     |    |       |     |    |       |        |        |        |
| Mean                           | 14.9    | 8.7               | 37.8  | 45.5 |     |       | 6.6 | 67.7 | 74.3  | p = NS | p = NS | p = NS |
| SD                             | 16.6    | 10.8              | 45.8  | 51.4 |     |       | 9.3 | 77.7 | 80.0  |        |        |        |
| Middle lobe                    |         |                   |       |     |    |       |     |    |       |        |        |        |
| Mean                           | 7.5     | 6.7               | 57.5  | 63.2 |     |       | 6.5 | 63.8 | 70.3  | p = NS | p = NS | p = NS |
| SD                             | 10.8    | 6.8               | 78.7  | 80.4 |     |       | 8.6 | 69.4 | 69.5  |        |        |        |
| Paired Student’s t test (upper vs middle) | p = 0.013 | p = NS | p = NS | p = NS | | | |                |        |        |        |

*The data from the PCP-positive and PCP-negative subjects are compared using unpaired Student’s t tests. PCP = \( Pneumocystis carinii \) pneumonia; BAL = bronchoalveolar lavage; Supe = BAL supernatant, CP = cell pellet of BAL; total = supernatant plus cell pellet.

Analysis

Pentamidine levels are reported in nanograms per milliliter of BAL; supernatant, cell pellet, and the total (supernatant plus cell pellet) values from the upper and middle lobes (Table 1). Levels were compared by paired analysis. The data were analyzed as supernatant, cell pellet, and total because a previous study has shown that cell pellet and supernatant levels are not always closely related.\(^11\) To compare levels between patient groups with documented PCP vs those negative for breakthrough pneumonia, independent Student’s t tests were used. In patients with documented PCP, linear regression analysis was applied to the relationship between pentamidine levels and \( P \) carinii counts for the upper lobe and for the middle lobe.

RESULTS

There were 32 patients in whose BAL fluid \( P \) carinii clusters were detectable (PCP-positive subjects) and 19 patients in whom \( P \) carinii was not detectable (PCP-negative subjects). The mean (± SD) age for the two groups was not different using Student’s t tests: PCP-positive subjects were 37.3 ± 7.4 years old; PCP-negative subjects were 33.4 ± 5.9 years old. The mean (± SD) duration of AP therapy and the time elapsed since last receiving AP for the two groups were not different using Student’s t tests: PCP-positive subjects receiving prophylaxis for 17.3 ± 9.5 months, 23.3 ± 21.7 days since last treated; PCP-negative subjects receiving prophylaxis for 15.2 ± 9.7 months, 31.6 ± 12.6 days since last treated. The diagnoses in the PCP-negative subjects were as follows: bacterial pneumonia (2 patients), cytomegalovirus (2 patients), and tracheobronchitis (15 patients). All of the PCP-negative subjects recovered without empiric anti-PCP therapy, and none had a diagnosis of PCP made in 6 weeks of follow-up observation. The mean (± SD) return of BAL fluid from the upper lobe was 47 ± 43 ml and from the middle lobe was 54.4 ± 12.5 ml (p = 0.011, paired Student’s t test).

Mean data on lobar pentamidine concentrations and \( P \) carinii cluster counts are listed in the table; pentamidine concentrations (in nanograms per milliliter) of
**Figure 1.** Individual data points (ng/ml of BAL fluid, mean±SD) are shown. The upper lobe samples are represented by circles and the middle lobes by squares. The PCP-positive subjects are shown by closed symbols and the PCP-negative patients by the open symbols.

BAL supernatant, BAL cell pellet, the total BAL pentamidine (supernatant plus cell pellet), as well as *P. carinii*, quantified as the number of clusters per 500 nucleated cells. Data are presented with the patients divided into those with and without PCP. Paired analysis (Student's *t* test) revealed no significant differences between total pentamidine (supernatant plus cell pellet) levels in the upper and middle lobes for either the PCP-positive subjects or the PCP-negative subjects. Individual data points for total pentamidine are shown in Figure 1. There is marked intersubject variation in levels in both the middle and upper lobes. This marked variation is a feature of both PCP-positive and PCP-negative patients. The analysis was repeated with total pentamidine subdivided into supernatant or cell pellet with similar findings.

Using unpaired Student's *t* tests, there are no significant differences in the concentrations of pentamidine in the upper lobes (supernatant or cell pellet or total pentamidine) between the PCP-positive and PCP-negative patients. Similarly, no differences were detected in middle lobe levels between the two groups.

For the PCP-positive patients, there is no correlation between lobar cluster counts and lobar pentamidine, using linear regression for either the upper lobe or the middle lobe (*R*=0). In Figure 2, the number of clusters of *P. carinii* is plotted against total pentamidine in BAL for the middle and upper lobes. The number of *P. carinii* clusters in the upper lobe was greater than in the middle lobe (mean ± SD: upper = 14.9 ± 16.6, middle = 7.5 ± 10.8, *p* = 0.013, paired Student's *t* test).

**Figure 2.** Pentamidine levels (ng/ml) (ordinate) are plotted against the *P. carinii* cluster counts (abscissa); data are shown for the upper lobe (circles) and for the middle lobe (squares). Using linear regression, correlations are not significant (upper lobe: *y*=17.1-0.0554-2x, *r*=0.17; middle lobe: *y*=8.8-0.0126x, *r*=0.09, *p*=NS).

**Discussion**

This study demonstrates that in patients receiving prophylaxis with AP, the concentrations of pentamidine in the BAL fluid from upper and middle lobes are not significantly different. The levels of pentamidine detected in the supernatant and cell pellet are similar to published values for middle lobe BAL supernatant and cell pellet pentamidine levels that were measured in electively studied patients at another institution. The latter patients had no clinical, microbiologic, or radiologic evidence of PCP and were studied prior to their scheduled monthly AP therapy.

The similarity in mean levels of pentamidine between lobes contrasts with previous studies that have shown that deposition of AP is relatively reduced in the apices of the lung. For example, Smaldone et al had demonstrated, using a radioaerosol technique, that the deposition of pentamidine was reduced in the upper portion of the lungs (mean ± SEM = 74 ± 0.5 percent) relative to the lower portion and that middle lobe BAL levels of pentamidine, obtained within 24 h of the deposition study, correlated with the dose deposited in the patients. The differences between the two studies may be due to the fact that the study by Smaldone et al involved a radioaerosol deposition study performed in patients who had not received previous therapy with pentamidine, while in the present study, the patients had received multiple doses of AP, which has a prolonged half-life. There was a small but statistically significant difference in return...
of BAL fluid between upper and middle lobes. In a previous study, however, we found that pentamidine concentration was not affected by volume of BAL fluid.\(^8\)

For the PCP-positive subjects, there was no correlation between the \textit{P carinii} counts and the level of pentamidine in either the upper lobe or the middle lobe. Further, this study demonstrates that when PCP-negative patients are compared with PCP-positive patients, the two groups have similar regional concentrations of pentamidine. These results are consistent with the findings of Smaldone et al\(^2\) who demonstrated, in a prospective study, that there were no significant differences in the whole lung dose of pentamidine deposited in patients with and without breakthrough PCP. In the latter study, considerable intersubject variability in whole lung deposition was demonstrated in both groups, an observation consistent with the wide range of BAL levels demonstrated by individual subjects within both groups in the present study. The PCP-negative patients in this study were patients who had respiratory symptoms but had no PCP clusters on BAL. It is unlikely that any of these patients had undiagnosed PCP because all of these subjects recovered without empirical anti-PCP therapy and none had a diagnosis of PCP made in 6 weeks of follow-up observation.

The demonstration of higher counts of \textit{P carinii} in the upper lobe is consistent with a study by Baughman and Dohn.\(^7\) They demonstrated that lavage of the apical segment of the right upper lobe yielded more \textit{P carinii} clusters than the middle lobe regardless of whether the patient had received AP. They also showed that patients presenting with radiographic patterns of upper lobe predominance were more likely to have higher \textit{P carinii} counts in the upper lobe BAL relative to middle lobe BAL. However, they reported that radiographic patterns of upper lobe predominance could occur in patients not receiving AP prophylaxis—a finding previously reported.\(^8\)

Jules Elysee et al\(^2\) demonstrated that patients who had been receiving AP had a higher incidence of \textit{P carinii} with radiographic patterns of upper lobe predominance whereas patients not receiving AP relapsed with a more diffuse radiographic pattern. Comparison with the present study is complicated by the fact that the patients studied by Jules-Elysee et al\(^2\) were treated with a nebulizer system that produces a more central pattern of pentamidine deposition than the nebulizer (Respigrad II) that was used in the present study. Nevertheless, in the present study, the absence of a relationship between cluster count and pentamidine level, the similarity in regional pentamidine levels between upper and middle lobes, and the similarity in pentamidine levels between the PCP-positive and PCP-negative groups indicate that the regional dose of pentamidine is not the determining factor as to whether AP prophylaxis will succeed or fail. Therefore, we would predict that breathing maneuvers and nebulizer modifications designed to improve upper lobe drug delivery would be unlikely to eliminate the problem of PCP breakthrough. Other causes should be considered. For example, drug resistance or changes in host defenses may be important.\(^13\) Cystic disease, which may be prominent in upper lobes,\(^8\) may prevent local penetration of drug and thus contribute to localized PCP breakthrough.

This study demonstrates that the upper and middle lobar concentrations of pentamidine are similar in PCP-positive and PCP-negative patients. Further, in patients with breakthrough \textit{P carinii} pneumonia, there is no correlation between lobar \textit{P carinii} cluster counts and pentamidine levels. We conclude that regional differences in pentamidine dose do not contribute significantly to failure of pentamidine prophylaxis.

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**REFERENCES**