Co-Trimoxazole Treatment of Two Fatal Cases of *Pneumocystis carinii* Pneumonia —Changes in Protozoal Morphology and Treatment Method—

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Summary

In two patients (one case of non-Hodgkin, mixed-type malignant lymphoma and one case of acute lymphatic leukemia) treated with co-trimoxazole for suspected *Pneumocystis carinii* pneumonia, cysts were demonstrated in the sputum after one day of treatment, and the patients died no longer viable four days of therapy. The morphology of cysts found in the pulmonary alveoli were examined by electron microscopy, and the treatment method was evaluated.

In patients with *P. carinii* pneumonia, the sputum is often non-productive, but examination for cysts should be performed adequately even after initiation of treatment, since expectoration of sputum becomes easier once treatment has been started.

In *P. carinii* found in pulmonary alveoli, fissuring of cell membranes were confirmed together with changes in the cytoplasm including the presence of intracystic bodies even after 4 days of treatment. Moreover, since lamellar-body-like structures such as are seen in pulmonary alveolar proteinosis were found in our cases, we surmised that active use of bronchoalveolar lavage to remove the material filling the alveoli is valuable not only for diagnostic purposes but also therapy of *P. carinii* pneumonia in which initiation of treatment has been delayed.

Introduction

Pneumocystis carinii pneumonia has recently drawn special attention as an infection occurring in patients with acquired immunodeficiency syndrome (AIDS).

Two patients with *P. carinii* pneumonia died after several days of treatment with co-trimoxazole. The morphology of cysts found in the pulmonary alveoli was investigated by electron microscopy, and we discussed the methods of treatment for cases in which initiation of treatment has been delayed. The results are described hereinafter.

Case Report

Case 1. A 51-year-old woman

In October of 1982, when she was 49 years old, she was admitted to a local hospital for surgery for a subarachnoid hemorrhage. When she was 50 years old, she noted a tumor in the left cervical region, and the tumor was diagnosed as a malignant lymphoma of non-Hodgkin, mixed-type based on the biopsy findings. Despite cobalt radiation therapy, no reduction in the site of tumor was seen, and she was admitted to our hospital on November 28, 1984. Findings of a CT-scan revealed swelling of the lymphnodes in the upper

Fig. 1 Case 1 (a 51-year-old woman), sputum smear preparation (Giemsa staining, $\times 500$)

mediastinum, tracheal bifurcation, spleen and cecum, and therefore combination therapy with anticancer agents was started from December 20. Although a fever began to develop February 23 (the 87th hospital day), chest roentgenographic findings revealed no abnormalities. However, culture of sputum yielded high amounts of *Haemophilus influenzae* and *Candida albicans*, treatment with cefmetazole was started. Even though the fever tended to fall, cefmetazole was discontinued because it was suspected of causing a rash which had developed. From February 27 (the 91st hospital day), a high fever appeared again. Cefotiam was administered, but with no improvement. Chest X-ray films taken on March 3 (the 95th hospital day) showed an abnormal shadow, chiefly involving the left hilum. These findings were interpreted as representing the pattern of interstitial pneumonia. Since dyspnea and cyanosis became predominant, *P. carinii* pneumonia was suspected and 12 tablets of co-trimoxazole (each tablet containing 80 mg of trimethoprim and 400 mg of sulfamethoxazole) per day in 3 divided doses was started from March 7 (the 99th hospital day). The co-trimoxazole treatment was combined with intravenous injection of γ -globulin at 2.5 g/day for 3 days. On the second day of co-trimoxazole treatment, cysts were detected in sputum smear preparations by Giemsa stain (Fig. 1). Chest roentgenograms revealed bilateral infiltration involving the whole left lung field. From these findings, it was surmised that absorption of co-trimoxazole was insufficient by the oral route. Therefore, oral administration was switched to intravenous administration, but the patient died on March 10 (the 102nd hospital day). Hypoxemia was not improved even after the patient was placed in on the oxygen tent since chest roentgenograms taken on the day of death revealed expansion of the shadow to encompass both lung fields.

Necropsy findings on the lung: Histologically, the pulmonary alveoli were filled with eosinophilic foamy materials and were surrounded by numerous histiocytes and small round cells. The foamy material stained positively with PAS staining and dark-brown with PAM staining. Thus, the patient was diagnosed as having had *P. carinii* pneumonia.

Electron microscopic examination revealed some relative changes among the cysts found in the alveoli. In thick-walled cysts, vacuolation of the cytoplasm or intracystic bodies (sporozoite) was noted (Fig. 2). On the other hand, thin-walled cysts (trophozoite) showed split cell membranes with loss of cytoplasm (Fig. 3). Scanning electron microscopic examination revealed masses of material containing cysts in the alveoli (Fig. 4).

Case 2. A 42-year-old woman

In April of 1984, she was admitted to our hospital with chief complaints of a low-grade fever, arthralgia, hemorrhagic tendency and leucocytosis. From the results of bone marrow puncture, the patient was

Fig. 2 Electron microscopic findings of cysts found in case 1 (a 51-year-old woman)

Fig. 3 Electron microscopic findings of *P. carinii* found in pulmonary alveoli in case 1 (a 51-year-old woman), showing trophozoites with split cell membranes and those containing no cytoplasm

Fig. 4 Scanning electron microscopic findings of the lung of case 1 (a 51-year-old woman)

diagnosed as having acute lymphatic leukemia (ALL). She was treated by combination therapy with antileukemic agents, showed improvement and was discharged in June of 1984. After that, she was readmitted in November for 20 days for a bone marrow examination. Even during the period when she came to our outpatient service, her condition was well-controlled. From the end of 1984, myeloblasts began to increase (75.8%), and this was accompanied by a decrease in muscle strength and numbness of the legs. She was admitted a third time on July 3, 1985, and marked anemia was noted. On the day of admission, a subarachnoid hemorrhage occurred, which was considered to be related to hemorrhagic diathesis due to ALL, and disseminated intravascular coagulation syndrome was also present as a complication. Various treatments resulted in improvement and her general condition was good. However, a myelogram revealed incomplete remission, with myeloblasts accounting for 20%. From February 17 (the 45th hospital day), a

Fig. 5 Case 2 (a 42-year-old woman). A cyst is seen in pulmonary alveolar space (arrow)

Fig. 7 Electron microscopic findings of cyst with divarication of cell membrane found in alveoli of case 2 (a 42-year-old woman)

Fig. 6 Electron microscopic findings of cyst with breaking of cell membrane found in alveoli of cases 2 (a 42-year-old woman)

Fig. 8 Lamellar-body-like substances (shown by an arrow) found in alveoli of case 2 (a 42-year-old woman)

fever in association with a decreasing tendency in the white blood cell count appeared, but the chest roentgenograms taken at this time revealed no abnormalities. Despite administration of various antibiotics and use of γ -globulin, she did not become afebrile. Moreover, blood and sputum were frequently cultured and were always negative, with no isolation of organisms likely to be the cause of her fever. Based on these results, we suspected *P. carinii* pneumonia or a fungal infection, and the treatment was further supplemented with 12 tablets of co-trimoxazole per day in 3 divided doses and 5-fluorocytosine from February 26 (the 54th hospital day). Sputum smears prepared (Giemsa stained) on the second day of administration of co-trimoxazole revealed the presence of cysts. A slight defervescent tendency appeared after administration of co-trimoxazole, but the chest roentgenograms revealed expansion of the shadow, which came to involve the whole lung fields. Thereafter, the general condition aggravated, and the patient died on the 59th hospital day.

Autopsy findings: There was diffuse interstitial pneumonia with hyaline membrane formation, intra-alveolar fibrinous material and foamy cell exudation, and electron microscopy revealed *P. carinii* in the pulmonary alveolar spaces (Fig. 5).

Among the cysts found in the pulmonary alveoli, some showed split cell membranes as seen in Fig. 6,

Fig. 9 Scanning electron microscopic findings of the lung of case 2 (a 42-year-old woman)

some showed changes in the cytoplasm including intracystic bodies and some remained in fibrinous forms with breaking and separation of the cell membranes (Fig. 7). The material filling the alveoli contained a number of lamellar-body-like structures such as are seen in pulmonary alveolar proteinosis were observed (Fig. 8). Examination by a scanning electron microscope revealed cell elements including cysts in the fibrous material which filled the alveoli (Fig. 9).

Discussion

When patients with AIDS develop *Pneumocystis carinii* pneumonia, the general condition aggravates rapidly and usually results in death. Moreover, when initiation of treatment is delayed, especially when treatment is started only after chest roentgenograms have revealed a generalised shadow involving the whole lung fields as in the 2 cases presented here, the prognosis is often poor.

In *P. carinii* pneumonia it is important to isolate the responsible pathogen, *P. carinii*, by *in vitro* culture as part of the laboratory studies, and detection of cysts is difficult since the patients do not produce much sputum. In view of these facts, some recent papers have described improvement in the diagnostic rate of this disease through the use of broncho-alveolar lavage, brushing or lung biopsy under bronchoscopic observation^{1)~3)}. However, as with our 2 cases, it may be possible to establish diagnosis if repeated examinations are performed using sputum obtained even after treatment has been started. It seems that expectoration of sputum may become easier after initiation of treatment. Similarly, some other papers have also suggested in the importance of examination of sputum⁴⁾⁵⁾. Pitchenik *et al.*⁴⁾ compared the detection rate of cysts in patients with AIDS or suspected AIDS between sputum samples obtained after 10 to 20 minutes of ultrasonic nebulization using 5% saline and samples obtained by bronchial washing, brush biopsy or transbronchial lung biopsy (TBLB) under bronchoscopic observation immediately after nebulization. They were able to establish a diagnosis of *P. carinii* pneumonia in 20 out of 40 cases, with a cyst detection rate of 55% for sputum samples, 53% for brush biopsy, 79% for bronchial washing and 90% for TBLB. Since there is no need to perform bronchoscopy when cysts can be detected from sputum and since TBLB under bronchoscopic observation does not give positive results in all cases, they emphasize the importance of examination of expectorated sputum. Use of an ultrasonic nebulizer is thought to make expectoration of sputum easier,

owing to penetration of the nebulized solution into the deep areas of the alveoli.

In treating these cases, co-trimoxazole or pentamidine is used. Because co-trimoxazole is less toxic, it is applied as the drug of first choice. However, Kovacs *et al.*⁶⁾ reported that adverse reactions to pentamidine were less frequent than generally expected, while the incidence of adverse reactions to co-trimoxazole was unexpectedly high. No great difference in efficacy has been observed between these two drugs⁷⁾. In this study, we used co-trimoxazole, but both patients died 4 days after starting treatment with this drug. This can be explained by the delay in initiation of treatment. The *Pneumocystis* detected in the pulmonary alveoli showed obvious changes due to the activity of co-trimoxazole. In thick-walled cysts, breaking and splitting of cell membranes were confirmed, and vacuolation of the cytoplasm or intracystic bodies were observed. Trophozoites showed splitting of the cell membrane, loss of cytoplasm, vacuolation and hyalization of nucleus. It is noteworthy that the number of trophozoites was very small, especially in case 2. This finding is very interesting in view of the fact that trophozoites greatly outnumber cysts in untreated cases. Co-trimoxazole is considered to be an antagonist of folic acid metabolism. To our knowledge, however, few or no papers have been published describing the results of following-up of changes caused in *P. carinii* by co-trimoxazole. The delay in initiation of treatment in our cases was very regrettable to us since the patients died even though striking changes in the cysts in the alveoli were seen after only 4 days of treatment. Regarding case 2, it is interesting that the presence of lamellar-body-like structures which are seen in pulmonary alveolar proteinosis (PAP), was confirmed. Although the etiology of PAP has not been fully elucidated, it appears that proliferation of type II alveolar cells, intraalveolar accumulation of debris from those proliferated cells and poor alveolar clearance by macrophages are responsible factors. Therefore, electron microscopic observation revealed the presence of destructed type II pneumocytes containing lamellar-bodies within the alveoli⁸⁾.

Bedrossian *et al.*⁹⁾ regarded PAP as a disease resulting from depression of secondary alveolar clearance associated with immunological suppression, and they claimed that a weaker inflammatory reaction was seen in the alveoli and therefore opportunistic infections are apt to develop. In fact, infections caused by fungi such as *Aspergillus* are often seen in PAP. There is a report of infantile cases of thymic aplasia complicated with PAP and *P. carinii* pneumonia¹⁰⁾. Nagai¹¹⁾ assumed that *P. carinii* was the causative organism of PAP since it was detected in the lungs of some patients who died from PAP. However, the morphology of lamellar-body-like structures observed in case 2 were different from that of damaged *P. carinii*. The foamy material which fills the alveoli in *P. carinii* pneumonia interferes with gas exchange in the alveol, causing hypoxemia. Thus, it is thought that in cases where disturbance of alveolar clearance is surmised the patients life might possibly be saved if this disturbance can be eliminated by some method. As with the cases presented here, when the material clogging the pulmonary alveoli cannot be expectorated despite remarkable changes in alveolar cysts even after only a short period of treatment, alveolar washing is valuable as both a diagnostic and a therapeutic method.

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Co-trimoxazole 治療中に死亡した *Pneumocystis carinii* 肺炎の 2 例 — 原虫の形態の変化および治療法について —

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Non-Hodgkin, mixed type の悪性リンパ腫 1 例および急性リンパ性白血病 1 例の計 2 例に、*Pneumocystis carinii* 肺炎を疑って co-trimoxazole を投与し、治療開始後喀出痰中に cyst を検出したが、治療 4 日後に死亡した。これらの症例の肺胞にみられた cyst の形態を電顕を用いて追求すると共に治療方法を検討した。

肺胞内の cyst は、僅か 4 日間の治療でも、intracystic body を含む原形質に変化がみられたほか、細胞膜の断裂・離開像が確認された。更に肺胞蛋白症の際にみられる lamellar-body 様物質を認めた。

P. carinii 肺炎では痰の喀出が少なく、痰中の

cyst の検出は難しいとされている。しかし治療により痰の喀出が比較的容易になるようで、治療開始後も cyst の検索を十分に行うべきである。

今回の症例では短期間の治療にもかかわらず、*P. carinii* に強い変化がみられたのに死亡したが、それは cyst を含む物質によって肺胞が充満され、ガス交換が障害されて著しい低酸素血症を招来したためと考えられた。したがって肺胞を充満する物質を、何らかの方法によって除去すれば、救命可能と推定された。ことに肺胞蛋白症におけると同様に、肺胞クリアランスの低下が想定され、治療開始が遅れた症例を完治させるには、化学療法のみでは不十分であり、肺胞洗浄などを合わせ行うことが、診断のためのみならず治療を行う上でも必要であろう。

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