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Respiratory Involvement in Polychlorinated Biphenyls Poisoning[1]

NOBUAKI SHIGEMATSU, SHUZO ISHIMARU, REIKO SAITO, TOGO IKEDA, KENICHI MATSUBA, KOTARO SUGIYAMA, AND YOSHITO MASUDA

The Research Institute for Diseases of the Chest, Kyushu University Faculty of Medicine, and Analytical Chemistry Laboratory, Daiichi College of Pharmaceutical Sciences, Fukuoka, 812, Japan

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Clinical, laboratory, and pathological findings on respiratory involvement in polychlorinated biphenyl (PCB) poisoning were studied in 401 patients and their pathological changes were produced in rats given PCBs orally. Respiratory symptoms included expectoration in 40% of the 289 nonsmoking patients with PCB poisoning and mild wheezing in 2%. The incidence and severity of the respiratory symptoms correlated well with the concentration of PCBs in the blood and sputa. Chest roentgenographic findings, pulmonary function tests, and pathological findings revealed bronchiolitis in many, and pneumonia or atelectasis in about one-tenth of the patients with reticulo-linear shadows. Peribronchiolar changes may be primarily due to either PCB poisoning or associated infection. Respiratory distress was often exacerbated by viral or bacterial infection persisting for more than a half year in about half of the patients examined. The IgA and IgM levels in the serum decreased considerably within 2 years after the onset of the disease and definite decreases in IgA levels may correlate well with the bacterial infection. PCBs found in sputa may have been present in association with lipid in type II cells of the lung (or with excretion from bronchial cells) and may have been phagocytosed in alveolar macrophages and may change their phagocytic function.

INTRODUCTION

In the summer of 1968 patients with polychlorinated biphenyl (PCB) poisoning began to appear throughout the western part of Japan. The cause was traced to the contamination of edible rice bran oil by PCBs used as a coolant (Kanechlor (KC) 400, the trade name for a mixture of PCBs) in the manufacturing process (27). Since then the disease has been called "Yusho," which means "the oil disease." Respiratory distress occurring in these patients improved gradually but persisted in most cases (26), especially in those with high blood concentrations of PCBs and with signs of chronically infected airways.

The accumulation of PCBs has recently been reported in mouse bronchial mucosa with dose dependence (3) and with structural requirements (5) of PCBs, but it has been stated that the biological significance of these observations is unknown. The purpose of this report is to present details of clinical, laboratory, and pathological findings of respiratory involvement in "Yusho" which have not been reported so far.

MATERIALS AND METHODS

Clinical and pathological examinations. Four hundred one patients with PCB poisoning form the basis of the present study. They were subjected to the follow-

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ing examinations: an assessment of subject symptoms, chest roentgenograms, pulmonary functions, microbial study of the sputum, and determination of immunoglobulin levels and PCB concentration in serum and sputum. A pathological study was done on the lungs from seven autopsy cases and from rats given PCBs, as described below.

Blood sampling and immunological and chemical analyses. Immunoglobulin levels were examined by radial single immunodiffusion (24) using Partigen (Behringwerke, West Germany). Immunoglobulin levels in 149 samples of serum out of 72 patients (59 adults and 13 children) from March 1970 through March 1972 and IgA in sputum from 9 adults were determined.

Rough estimates of the PCB concentrations in sputum samples, averaging 30 g, collected from the patients before 1972 were obtained by comparing total peak heights on gas chromatograms (18). Sputa collected after 1975 from 20 patients and averaging 40 g were semiquantitatively analyzed using the Japanese standard analytical method for PCB (20). Since 1973, about 10 ml of blood taken from each patient and each of 37 healthy volunteers has been analyzed by the latter method for PCB.

Animal experiments. Male rats of the SD strain, weighing about 200 g, were given, by gastric intubation, 25mg of KC 400 in 0.5 ml of edible oil once (Group I PCBs) and only 0.5 ml of oil (Group I control) and the same dose four times per week (Group II PCBs and control). Groups of five animals were killed by cervical dislocation at 2, 7, 14, and 28 days after the last ingestion. Tissues were prepared for light and electron microscopy.

RESULTS

Respiratory symptoms and their relation to PCB concentration in blood. The ages of the 401 patients with Yusho were distributed between newborn and 80 years and the sex distribution was approximately equal. Respiratory symptoms were cough, expectoration, and wheezing, the former two appearing with skin eruptions and the latter ensuing several months later. Expectoration was nonvisceid in those without airway infection, comprising 40% of the 289 nonsmoking patients and mild wheezing was noted in 2%.

In one case with bilateral cicatrical thickening of pleura due to tuberculosis which healed in 1943, respiratory insufficiency and CO_2 narcosis have developed three times since 1970 because of airflow obstruction exacerbated by bacterial infection. His PCB concentration in the blood and sputum was high, 27 and 8 ppb, respectively, in 1975. The incidence of respiratory symptoms correlated well with the concentration of PCBs in the blood (this trend was significant, $p < 0.05$) (1), but not with the pattern of PCBs (the latter being noted in Table 1).

Chest X-ray and laboratory findings. On chest roentgenograms 35% of the patients revealed reticulo-linear shadows, upon which acinar, patchy (bronchopneumonic), or atelectatic shadows were superimposed in about 10%. These roentgenographic findings did not correlate well with the severity of the dermal eruption (25). The results of pulmonary function tests in 12 nonsmoking patients are presented in Table 2. Vital capacity (VC) and the ratio FEV_1/FVC were almost normal but arterial oxygen tension (pO_2) decreased in 8 and maximal expiratory flow at 50 and 25% of vital capacity ($\dot{V}_{max\ 50}$ and $\dot{V}_{max\ 25}$) showed mild decreases

TABLE 1

RESPIRATORY SYMPTOMS AND POLYCHLORINATED BIPHENYLS IN BLOOD

PCB pattern	PCB concentration (ppb)	Respiratory symptoms +	Percentage	Total
A	1–4	21 (2)[a]	8 (7)	27.6	29 (9)
	5–9	21 (2)	19 (12)	47.5	40 (14)
	10–19	7 (4)	7	50.0	14 (7)
	20–31	2	4	66.7	6
B	1–4	5 (3)	3 (2)	37.5	8 (5)
	5–9	3 (2)	2	40.0	5 (2)
	10–19		2 (1)	100.0	2 (1)
B + C	1–4	15 (5)	13[b] (9)	46.4	28 (14)
	5–9	5 (1)	3	37.5	8 (1)
C	1–4	87 (14)	50[c] (35)	36.5	137 (49)
	5–9	7 (2)	5 (8)	41.7	12 (10)
Total		173 (35)	119 (76)	40.4	289 (111)

[a] The numbers in parentheses represent patients with smoking habit excluded.

[b] Four out of the thirteen patients had a past history of asthma.

[c] Two out of the fifty patients had a past history of asthma.

in about half of the patients; in the latter both inspiratory and expiratory rhonchi were audible at all times.

Serial bacteriological examination of sputa in 12 nonsmoking patients with respiratory distress for over 2 years revealed the presence of *Staphylococcus au-*

TABLE 2

PULMONARY FUNCTION TESTS IN 12 NONSMOKING PATIENTS[a] WITH RESTRICTIVE-TYPE SYNDROMES ABOUT 1 YEAR AFTER ONSET OF RESPIRATORY DISTRESS AND AT FOLLOW-UP

Measurement		1970	1973–1974
VC/predicted VC (%)	>100	5	6
	>90	1	5
	>80		1
FEV_1/VC (%)	>80	3	9
	>75	3	3
$\dot{V}_{max.\,50}$ (liters/sec)	>4.0		6
	>2.0		6
$\dot{V}_{max.\,25}$ (liters/sec)	>1.5		5
	>1.0		5
	>0.7		2
$P_{a}O_2$ (mm Hg)	>85	3	4
	>70	2	7
	>60	1	1

[a] Ages were distributed between 30 and 49 years, and half of them noticed first in 1970 that their respiratory distress might be related to the poisoning.

reus, *Escherichia coli*, *Pseudomonas*, or *Hemophilus*. The bacteria were found persisting for more than 1 year in three cases and less than 1 year in two cases. In these five cases the PCB concentrations were over 10 ppb in the blood and 3 ppb in the sputa, while the cases with PCB concentrations under 10 ppb were affected without persistence. Therefore, chronically infected airways seemed to be present in about half of the patients with respiratory distress, and further, it was often exacerbated by viral infection with the appearance of inflammatory symptoms in the upper airways.

Case presentation. As a typical case of Yusho involved in respiratory disorders, the 46-year-old wife of a patient with respiratory insufficiency described above had complained of expectoration since April 1968 and mild wheezing since July 1968, complicated by dermal chloracne-like eruptions and black pigmentation, hepatomegaly, and the like (23) since April 1968. Chest roentgenograms showed bilateral reticulo-linear and micronodular shadows. In a pulmonary function test performed in 1973, the following results were found: VC, 2470 ml (92%); FEV_1/FVC, 76%; $\dot{V}_{max\ 50}$, 2.6 liters/sec; $\dot{V}_{max\ 25}$, 0.7 liters/sec; pO_2, 72 mm Hg; pCO_2, 37 mm Hg. Abnormality in the flow–volume curve and blood oxygen tension suggests that she had been involved in small airway disease. In microbial examination of sputum bacteria were detected, i.e., *Staphylococcus aureus* in each culture in 1970 and *Hemophilus* in 1975. Rhonchi were improved after appropriate chemotherapy. The blood PCB content of this patient was 12 ppb and pattern A in both 1972 and 1975 and the PCB content in the sputum was 3.3 ppb and pattern B.

Immunoglobulin levels. The IgA and IgM levels in the serum decreased and the IgG level increased in 1970 but all were restored to normal in 1972, except in three cases (one adult and two children) where the IgA level remained low (24). Though no statistically significant relationship between IgA levels and clinical symptoms was detected, IgA levels of less than 100 mg/100 ml were found in 5 of 29 cases with respiratory symptoms but none in 24 cases without. The IgM levels were significantly lower in patients with severe dermatological symptoms (grade IV, (9), while the IgA levels in sputum were not low (24).

PCBs in blood, sputum, and fat tissue. Gas chromatograms of PCBs in blood were reported in a separate paper (20). In the gas chromatograms, the peak appeared immediately after 2,2-bis(4-chlorophenyl)-1,1-dichloroethylene (DDE) was low and the fifth major peak with DDE was high in the majority of the patients (pattern A); the reverse was present in the peaks of normal persons (pattern C). Pattern B resembled pattern A but was not as pronounced. The sputa collected from December 1969 to July 1970 were analyzed for PCBs by gas chromatography. The definite PCB peaks with late retention times were always detected in the sputa collected before May 1970 and apparently were lower in those after June 1970. In sputa collected after 1975, about the same level of PCB concentration was found as in those after June 1970 and was about one-third to one-tenth of the blood concentration. The PCB peaks of the same pattern demonstrable in the fat tissue of autopsy patients in 1969 were over 100-fold greater than those in sputa collected at about the same time (26).

Pathological study. Pathological study of lung specimens from seven autopsy patients disclosed lymphocyte infiltration in bronchial and bronchiolar walls and

macrophage infiltration in alveoli, particularly around bronchioles associated with alveolar collapse in four at the age of 13 to 48 (Fig. 1). Focal hemorrhage and/or pulmonary edema and pleural and pericardial effusion or adhesion were also detected in three patients who died within 4 to 12 months after the onset of the poisoning (14, 15) (Fig. 2). Marked hyperemia, atelectasis, and alveolar hemorrhage were noted in a stillborn case (13).

In rat lungs peribronchial and peribronchiolar cell infiltrations, especially the latter, were observed (Fig. 3). Electron microscopic studies in Group II PCBs revealed large lipid vacuoles and altered lamellar bodies or lysosomes in a type II alveolar cell and alveolar macrophage (Fig. 4). These pathological changes were most marked at a week after the last ingestion of PCBs and more intense in the Group II PCBs than in Group I PCBs.

DISCUSSION

The cause and clinical symptoms of PCB poisoning (Yusho). In February 1968, leakage of PCBs used as a coolant in the manufacturing process caused the contamination of edible rice bran oil which was distributed mainly in the western part of Japan (19). Shortly thereafter unusual skin lesions were encountered (9). A certain but definite dose–effect relationship was noted between the amount of oil consumed and the clinical grade of dermal eruption (31). The average amount of oil consumed was 800 ml per person (31). The oil contained, as later found by chemical and activation analyses, 1000 ppm of PCBs (22) and caused a heavy poisoning affecting over 2000 persons.

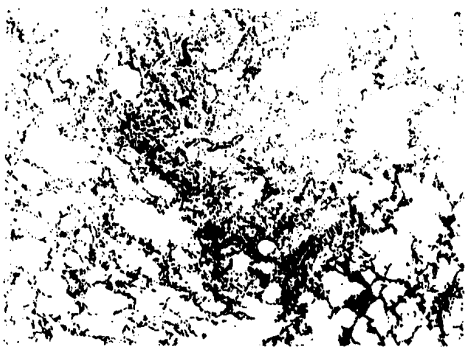

FIG. 1. Lung tissue from an autopsy case with PCB poisoning. Death was caused by acute cardiac insufficiency 4 years after the onset of the disease at the age of 46. Chronic inflammatory cell infiltration and productive changes are displayed in and around the bronchioles, and edema and macrophages are seen in the alveoli. × 42

FIG. 2. Lung tissue from another autopsy case with PCB poisoning. Death was caused by acute cardiac insufficiency 1 year after the onset of the disease at the age of 13. Lymphocyte and macrophage infiltration are seen in bronchiolar walls, and hemorrhage and macrophages are present in the alveoli. × 92.

The other symptoms in Yusho, which varied a great deal, are given in the tables (16, 19). Respiratory symptoms have been omitted because of unawareness of their relationship to PCB poisoning at that time. Although PCBs are lipid soluble and vigorous lipid metabolism is known to be present in the lung (11, 12), no respiratory involvement in PCB poisoning has been reported until now, apart from pleuritis occurring in chlorparaffin poisoning (12) and in chloracne cases (2).

Concentration and distribution of PCBs in tissue, blood, and sputum. The determination of PCBs in tissues from autopsy patients showed that the total amount present in the lung was about 1/15th to 1/40th of that present in the skin or in fat tissue (18). The distribution of PCBs in animals has been studied extensively (4–6). In rats the distribution of KC-400 administered orally was highest in the skin, adipose tissue, and liver, followed by plasma. It was somewhat lower in the lung (30). Tetrachlorobiphenyl, the major constituent of KC-400, is almost completely eliminated from the body in 3 to 4 weeks, but penta- and hexa-analogs, although they are minor components, are still retained after 9 to 10 weeks, persisting most tenaciously in the skin (29). As mentioned above, PCB concentrations in the fat tissue of patients (20) is much higher than that in blood or sputa. These different PCB concentrations imply that PCBs deposited in the fat tissue are being removed slowly and then appear in blood and sputum.

Recent studies in mice showed that a number of discrete PCBs were not taken up by the bronchi but were distributed very evenly throughout the lung parenchyma (4). However, the author also showed specific dose dependence (3) and structural requirements (5) of PCBs for accumulation in the mouse bronchial mucosa.

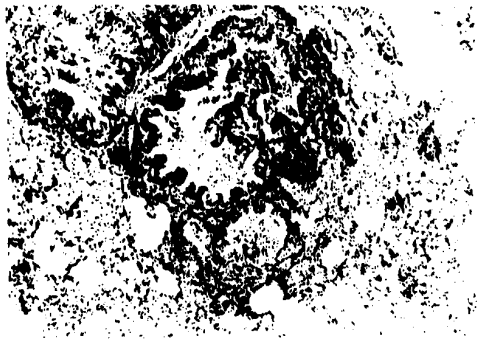

FIG. 3. Lung tissue from rat administered PCBs once a week for 4 weeks. Peribronchiolar lymphocyte and macrophage infiltration are seen conspicuously and macrophages are found to a lesser extent in the alveoli. Focal hemorrhages are also seen in the alveoli. × 95.

Pathophysiological changes. Pathological findings in the respiratory system in seven autopsy cases with Yusho consist primarily of macrophage infiltration and focal atelectasis in alveolar spaces. Alveolar collapse around bronchioles may result from disturbances of lipid metabolism in the lung, as is observed from measurements of the surface activity of lung extracts (25). Peribronchiolar changes may be primarily due either to PCB poisoning or to associated infection. Roentgenographic findings and altered pulmonary function correlate well with these pathological findings.

Focal hemorrhage and/or edema and pleural and pericardial effusion were mainly observed in the patients who died within 1 year after the onset of the disease. In February 1968, a large number of chickens died when fed with fodder mixed with oil from the same company. The feeding of Kanechlor 400 at a 0.04% level resulted in labored respiration and distended abdominal cavity, similar to chick edema (7, 10).

Alterations in the defense mechanisms of the respiratory tract. Respiratory distress is often exacerbated by viral or bacterial infection. In the latter, Gram-negative bacilli were often detected and found to persist in about half of the cases examined.

Factors involved in altering the defensive mechanisms of the respiratory tract include the clearance of PCBs, which is not known in detail. Follicle atrophy in the spleen (7, 14) or spleen atrophy (17) has been noted and in guinea pigs only the humoral immune response was depressed (28). In the patients with PCB poisoning IgA and IgM levels in serum apparently decreased for 2 years after the onset of the disease, but they were restored to normal in most cases in spite of the persistence of respiratory symptoms.

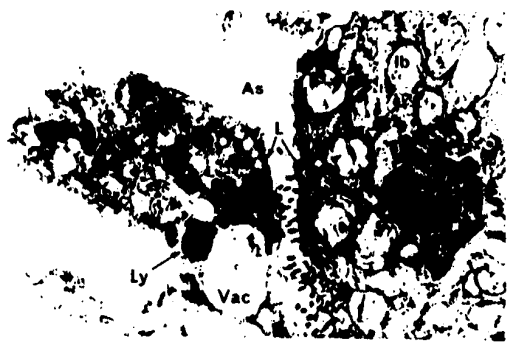

FIG. 4. Lung tissue from rat prepared for Fig. 4 fixed by Dermer's method (6). Note the attenuated lamellar bodies and decreased osmiophilia in a type II cell, and numerous dark bodies (lysosomes) and phagocytosed lipids in a macrophage. As, alveolar space; Ib, inclusion body; L, lipid; Ly, lysosome; Vac, vacuole. 4000.

Furthermore, the incidence of respiratory symptoms correlates well with the concentration of PCB. In sputum, PCBs may have been present in association with excretion from bronchial cells and/or with lipid in type II cells of the lung, phagocytosed in alveolar macrophages, and expectorated. PCB is a labilizer of the lysosomal membranes *in vitro* (8) and it resulted in low acid phosphatase activity in alveolar macrophages (25). These findings may imply its decreased phagocytic function.

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