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https://doi.org/10.15017/7323632

出版情報：福岡醫學雜誌. 86 (5), pp.261-266, 1995-05-25. 福岡医学会
バージョン：
権利関係：

Effect of Polychlorinated Biphenyls and Polychlorinated Dibenzofurans on Leukocyte in Peripheral Blood and Bronchoalveolar Lavage Fluid

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Abstract In order to investigate immunological abnormalities induced by polychlorinated biphenyls and polychlorinated dibenzofurans, we have performed bronchoalveolar lavage in the rats given polychlorinated biphenyls and polychlorinated dibenzofurans. We have administrated 5.0 mg of polychlorinated biphenyls or 0.5 mg of polychlorinated dibenzofurans to Sprague-Dawley rats intraperitoneally. Four weeks after the administration, mild necrosis of bronchiolar Clara cells and mild edema associated with chronic inflammatory infiltration in the alveoli were seen in both groups. In the peripheral blood, percentage of T-lymphocyte, helper T-cell and suppressor T-cell decreased significantly both in polychlorinated biphenyls and polychlorinated dibenzofurans given rats. On the other hand, in the bronchoalveolar lavage fluid, percentage of T-cell increased only in polychlorinated dibenzofurans given rats and percentage of suppressor T-cell increased in both groups. O_2^- release by alveolar macrophage increased significantly both when stimulated with wheat germ lectin and with phorbol myristate acetate. These results indicate that immunological alternation may be different between peripheral blood and respiratory system as one of the target organs of these chemicals. Further examination is needed for the analysis of immunological abnormalities in the target organs of polychlorinated biphenyls and polychlorinated dibenzofurans poisoning.

Introduction

"Yusho" is a mass food poisoning caused by ingestion of a commercial brand of rice oil contaminated with polychlorinated biphenyls (PCBs) and their related compounds. The major causal agents responsible for the early as well as late clinical manifestations of Yusho are now generally thought to be polychlorinated dibenzofurans (PCDFs)[7].

One of the target organs of PCBs and PCDFs is the respiratory system. Cough, sputum expectoration and wheezing were the major identifiable symptoms of Yusho, and respiratory distress was often exacerbated by viral or bacterial infection[13]. Animal experimental model suggests that PCDFs rather than PCBs cause pathologic changes in respiratory system; necrosis of the bronchiolar Clara cells and inflammation of alveoli[12)13]. In addition, there are several reports suggesting immunologic disorders both in patients with Yusho and Yu-Cheng, and in animals given PCBs and their related compounds[8)9)12)14)16]. However, there are few reports on the immunological change of respiratory system in spite of emphasis of respiratory involvement in patients with Yusho and Yu-Cheng. We have performed bronchoalveolar lavage in the rats given PCBs or PCDFs in order to analyze immune disorder of respiratory system.

Materials and Methods

1) Animal Experiments

Female rats of the Sprague-Dawley strain, 8-week of birth, were given 5.0 mg of PCBs

(Kanechlor 400) in 2 ml of corn oil (PCBs group), 0.5 mg of PCDFs in 2 ml of oil (PCDFs group) or only 2 ml of oil (control group) intraperitoneally. Four weeks after the administration, each group of the animals was anesthetized with ether and peripheral blood was collected from left ventricle using a heparinized syringe. White blood cells were counted with hemocytometer and lymphocyte count was determined by May-Giemsa stain. Lung tissues were prepared for light microscopy. Bronchoalveolar lavage was performed 2 times with PBS (calcium-and magnesium-free phosphate-buffered saline) after placing intravenous catheter in the trachea. Aliquots of the lavage fluid were divided for cell count, assay of surface marker of lymphocytes and determination of O_2^- release.

2) Analysis of Surface Markers of Lymphocytes

The monoclonal antibodies used were MAS 010 for pan T-cell, MAS 1131 for helper T-cell and MAS 041 for suppressor T-cell, respectively[1)17)]. The surface marker of lymphocytes were measured as described[1)] with minor modifications. In brief, peripheral blood and bronchoalveolar lavage fluid were incubated at 4°C for 45 min with saturating concentrations of each antibody. After washing, the cells were incubated with fluorescein-conjugated rabbit anti-mouse immunoglobulin and washed again. After removal of red blood cells with sodium ammonite, cell surface markers were examined in the FACS.

3) Production of by O_2^- alveolar macrophage

The release of O_2^- was assayed by the previously reported method, the superoxide dismutase-inhibitale reduction of cytochrome c, with minor modifications[15)]. In brief, aliquot of the lavage fluid was centrifuged at 220 x *g* for 10 min at 4°C. Cell pellets were incubated at 37 °C for 7 min after being suspended in 0.83% NH_4Cl and 170 mM Tris-HCl (9 : 1, vol/vol) at pH 7.5 for hemolysis of red blood cells. The cells were resuspended in RPMI 1640 medium (Sigma, St Louis, USA) containing 10% heat-inactivated fetal calf serum and poured into plastic petri dishes and incubated for 1 hour at 37°C followed by washing with phosphate-buffered saline to remove nonadherent cells and debris. The cells (4×10^5 cells) were suspended in 5 mM Hepes buffer (pH 7.4) containing 160 μM cytochrome c and 1 mM $CaCl_2$. After preincubation for 5 min at 37°C, the cells were first stimulated with cytochalasin E (10 μg/ml), followed in 3 min by wheat germ lectin (100 μg/ml) or phorbol myristate acetate (2.5 μg/ml). The change in absorbancy at 550 nm in reference to 540 nm was followed on a recorder by

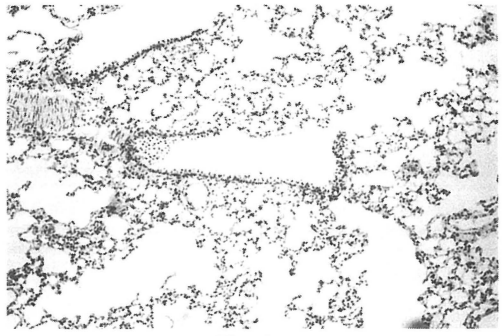

Fig. 1a

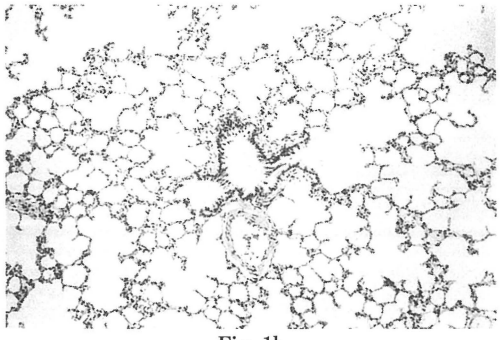

Fig. 1b

Tissue from lungs of rats given PCDFs (Fig. 1a). Clara cells are hyperplastic but more scarcely seen in terminal bronchiole, and mild edema with chronic inflammatory cell infiltration were seen in alveoli, compared with controls (Fig. 1b).

using a dual wave length spectrophotometer (Hitachi 556, Tokyo, Japan) and converted to superoxide release with a molar absorption coefficient of the reduced minus the oxidized cytochrome c as $19.1\times10^{3}M^{-1}\times cm^{-1}$.

Results

1) Pathologic changes in the lung

In rat lungs given PCDFs, Clara cells were hyperplastic but more scarcely seen in terminal bronchiole, and mild edema with chronic inflammatory cell infiltration were seen in alveoli, compared with controls (Fig. 1). In rat lungs given PCBs, these pathologic changes were more mild than those given PCDFs.

2) Effect of PCBs and PCDFs on Peripheral Blood

The changes of peripheral white blood in the rats given PCBs, PCDFs and oil alone as a control are summarized in table 1. Total count of white blood cells and lymphocytes tended to decreased in the rats given PCBs and PCDFs. Percent T-lymphocyte, helper T-cell and suppressor T-cell decreased significantly in both PCBs and PCDFs given rats, although the ratios of helper T-cell to suppressor T-cell were not different significantly in both groups.

3) Effect of PCBs and PCDFs on bronchoalveolar lavage fluid

In each procedure, recovery of the lavage fluid was more than 90%, and viable cells determined by trypan blue staining were more than 95%. The percentage of lymphocytes in the lavage fluid decreased both in PCBs and PCDFs given rats without significant difference. The percentage of T-cell significantly increased only in the lavage fluid of PCDFs given rats. Measurement of T-cell subsets revealed that percentage of suppressor T-cell increased significantly in both group, but that of helper T-cell was not different. The ratio of helper T-cells to suppressor T-cells was 0.79 for control,

Table 1 Effect of administration of PCBs and PCDFs on peripheral white blood cells.

Group	Control	PCBs	PCDFs
WBC (μl)	6783±2417	6317±2348	5333±1571
lymphocyte (μl)	6391±2241	5646±2446	4818±1349
% T-cell	66.5±12.2	48.0±16.2*	47.7±12.1*
% Helper T	47.5±6.9	33.7±8.2*	33.7±7.2*
% Suppressor T	29.1±5.2	19.0±7.1*	19.8±6.7*
ratio	1.67±0.11	1.91±0.30	1.78±0.13

Each value represents the mean ± S. D. of six rats.
*Significantly different from the control ($P<0.05$).

Table 2 Effect of administration of PCBs and PCDFs on bronchoalveolar lavage fluid.

Group	Control	PCBs	PCDFs
% lymphocytes	71.9±17.7	52.0±7.56	51.2±15.4
% T-cell	8.80±1.38	8.00±2.58	12.85±15.4*
% Helper T	5.20±2.63	6.20±2.58	5.73±1.23
% Suppressor T	6.43±1.31	9.03±1.50*	11.10±3.25*
H/S Ratio	0.79±0.36	0.67±0.24	0.54±0.10

Each value represents the mean ± S. D. of four rats.
*Significantly different from the control ($P<0.05$).

Table 3 Effects of administration of PCBs and PCDFs on superoxide producing activities of alveolar macrophages.

Production of O_2^- (nmol/min/2×10^5 cells)			
trigger	control	PCBs	PCDFs
WGA	0.14±0.05	0.31±0.07†	0.31±0.07*
PMA	0.13±0.05	0.29±0.06*	0.34±0.07*

Each value represents the mean ± S. D. of four rats.
*Significantly different from the control ($P<0.01$).
† Significantly different from the control ($P<0.05$).

0.67 for PCBs group, and 0.54 for PCDFs group. However, the difference was not statistically significant (table 2).

4) Effect of PCBs and PCDFs on production of O_2^- by alveolar macrophage

Release of O_2^- by alveolar macrophage is shown in table 3. When wheat germ lectin was used as a trigger of O_2^- release, O_2^- production increased significantly both in the rats given PCBs and PCDFs. Phorbol myristate acetate also stimulated O_2^- production with a statistical significance. There was no statistically significant difference in O_2^- production by alveolar macrophage between PCBs group and PCDFs group.

Discussion

The respiratory symptoms as well as skin seem to be strongly associated with higher blood PCB level and to be pathognomonic for Yusho, even twenty years after exposure[2]. The major causal agents responsible for clinical manifestations of Yusho are generally thought to be PCDFs[7]. In rats given PCBs and PCDFs by gastric intubation, necrosis of Clara cells, mild edema and vascular congestion in the lung were induced[12]. In this experiment, we adopted single administration of these chemicals by intraperitoneal injection in order to minimize the chance of infection and to give precise dose. Similar morphological changes were induced by intraperitoneal administration of PCBs and PCDFs, while 0.5 mg of PCDFs caused more severe pathologic chances than 5 mg of PCBs.

In patients with Yu-Cheng, various kinds of immunological abnormalities are reported[9]; decrease of serum IgA and IgM, negative skin test to tuberculin and to streptokinase/streptodornase antigen, decrease of T-cells and Tμ-cells (helper T-cells) in peripheral blood, the increased response of lymphocytes to phytohemagglutinin, to pokeweed mitogen and to tuberculin. In animal experiments, decrease of gammaglobulin levels[8)14)16)] and decrease of skin sensitivity to tuberculin[14] and to sheep red blood cell[3] has been noted. In our experiment, percentage of T-cell, helper T-cell and suppressor T-cell in peripheral blood were significantly decreased both in PCBs and PCDFs given rats. Clinical observation of the patients with Yu-Cheng revealed that T-cell and helper T-cell (but not suppressor T-cell) decreased in peripheral blood[9]. Our former report revealed that T-cell (Thy-1^+ cell) and helper T-cell (Lyt-1^+ cell) decreased in mice given PCDFs[11]. The discrepancy between thses former reports and the experiments using rats may resulted from difference of species, exposure period to the chemicals (one year vs. 4 weeks), difference of assay system or antibodies for lymphocyte subsets, although the details are not known. However, there are general accordance that abnormalities of T-cell and helper T-cell are seen in an early stage of this poisoning.

There are few reports on the immunological changes of respiratory system, although respiratory involvement in PCBs and PCDFs poisoning was reported. Bronchoalveolar lavage was performed in order to analyze lymphocyte subsets of lavage fluid and O_2^- generation by alveolar macrophage. Although a mild decrease of lymphocytes in the lavage fluid was seen in both groups, significant increase of percent T-cell was seen only in PCDFs given rats. The results of analysis of T-cell subset in the lavage fluid were generally opposite to those in peripheral blood; increase of percent suppressor T-cell and decrease of helper T-

cell/suppressor T-cell ratio. The discrepancy between systemic immune system and respiratory immune system is seen in several immunological disorders involving lung; In sarcoidosis, hypersensitivity pneumonitis and chronic berylliosis, systemic cell-mediated immunity is lowered in spite of enhanced immunity in the involved organs. Although the mechanism may be different between these diseases and PCBs and PCDFs poisoning, immunological abnormalities in target organs may not always reflect systemic immune function. In such a sense, investigation of abnormalities in target organ would be important for the better understanding of this poisoning.

Contrary to our expectations, increased O_2^- generation by alveolar macrophages was seen in both PCBs and PCDFs given rats. O_2^- is believed to injure cells by oxidizing cell membrane lipids and enhancing the permeability of lung vessels[10]. Pathological study of lung specimens from autopsy patients disclosed pulmonary hemorrhage and pulmonary edema[4)5)6)]. In our experimental system, mild inflammation of the alveoli was seen by the administration of PCBs and PCDFs. Therefore, in PCBs and PCDFs given rats, increase of O_2^- producing activities by alveolar macrophage might contribute to the pathogenesis of interstitial changes of the lung.

Acknowledgments

We thank Yoshito Masuda (Daiichi College of Pharmaceutical Sciences) for generous supply of PCBs and PCDFs and for valuable discussion.

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(Received for publication March 22, 1995)

(和文抄録)

末梢血ならびに気管支肺胞洗浄液中の白血球におけるPCBsとPCDFsの影響

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PCBsおよびPCDFsによって引き起こされる免疫系の異常を検討するために，PCBsならびにPCDFs投与ラットに気管支肺胞洗浄を施行した．5.0 mgのPCBsまたは0.5 mgのPCDFsをSprague-Dawley ratの腹腔内に投与した．投与4週後，細気管支クララ細胞の軽度の壊死性変化と慢性炎症細胞浸潤を伴った軽度の間質の浮腫が両群にみられた．末梢血では，T細胞，ヘルパーT細胞，サプレッサーT細胞の比率が有意に低下していた．一方，気管支肺胞洗浄液中では，PCDFs投与群にT細胞比率の上昇がみられ，サプレッサーT細胞の増加が両群でみられた．肺胞マクロファージによるO_2^-産生は，wheat germ lect in刺激でも，phorbol myristate acetate刺激でも有意に増加していた．これらの結果は，免疫系の変動は，末梢血とこれらの化学物質の標的臓器とでは異なることを示唆している．PCBs，PCDFsの標的臓器における免疫異常の解析をさらに進める必要性があろう．