HEALTH EFFECTS OF CHRONIC EXPOSURE TO POLYCHLORINATED DIBENZO-P-DIOXINS, DIBENZOFURANS AND COPLANAR PCB AROUND MUNICIPAL WASTE INCINERATORS

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Introduction

A national survey of polychlorinated dibenzo-p-dioxins (PCDD) and dibenzofurans (PCDF) in emission gases from municipal waste incinerators in 1997 revealed that the several factories emitted excess amount of PCDD/PCDF. We reported the results of 92 incinerator workers in DIOXIN'99, in which blood dioxin levels ranged from 13.3 to 805.8 pg TEQ/g lipid. It caused serious social problems among residents around the incinerators, so the Ministry of Health and Welfare started health screening for the residents in 4 contaminated areas and 4 control areas.

Subjects and Methods

Randomly selected 25 residents in each area, aged 30s and 40s, who had lived more than 10 years within 2 km of the municipal incinerator (area A) or apart more than 5 km from any incinerator (area B), were explained the purpose and methods of the study, and each gave a written informed consent. Final number of subjects in area A was 81, in area B was 95, totaled was 176.

A preliminary health check-up was performed by a physician if the subjects would allow collection of 130 ml blood for dioxin and laboratory analysis, and 96 males and 80 females remained as subjects for this study. Height, body weight, and blood pressure were measured, and the percentage body fat was calculated from body mass index according to the standard equation. Life habits and dietary habits were collected by questionnaire and checked by trained dieticians. The questionnaire included dietary habits, smoking and drinking habits, residential and work environment, physical activity, past history of diseases and treatments, reproductive history, etc.

Blood was collected into a 200 ml transfusion bag containing heparin sodium solution (SH-207-Terumo, Japan). About 30 ml of blood was divided to tubes to perform peripheral blood tests, such as RBC, WBC, and platelet counts, and hematocrit, and blood chemistry studies, such as determination of AST(GOT), ALT(GPT), gamma-GTP, LDH, ALP, LAP, CPK, amylase, total cholesterol, HDL-cholesterol, triacylglycerol, total protein, albumin, total bilirubin, blood urea nitrogen, creatinine, uric acid, glucose, creatine phosphokinase, sodium, potassium, calcium, iron, and inorganic phosphate. These tests were performed by the Serum Research Laboratory (Tokyo).

As immunologic markers, T lymphocytes subsets determined by surface antigens, such as CD3, CD4, CD8, and CD4/CD8 ratio, were also measured. NK activity was measured by surface antigen (CD56), and natural killer cell activity was determined against K562 cells. Stimulation by PHA and Con A was also applied.

Blood PCDD/PCDF/Co-PCB was measured by a modification of Patterson's method.

Lipids were extracted from 80-100 g of whole blood with a solution of 30 ml saturated ammonium sulfate and 80 ml of ethanol:hexane (1:3) solution after the addition of an internal standard of ^{13}C- labeled mixed dioxin congener solution, which contained 30 pg of $^{13}C_{12}$-PCDDs, $^{13}C_{12}$-PCDFs, and $^{13}C_{12}$-Co-PCBs, except OCDD (60 pg)(Wellington Isotope Laboratories, Massachusetts, USA)). All solvents were of dioxin analysis level. Pooled hexane layers were condensed, washed with distilled water, treated with anhydrous sodium sulfate, and evaporated to dryness, and lipid weight was measured.

Clean up was achieved by a multilayer silica column with 44% sulfuric acid and 33% potassium hydroxide, and an activated carbon column. Analysis of PCDD/PCDF/Co-PCB was carried out by gas chromatography-high resolution mass spectrometry (GC-MS). The analytical conditions were as follows: gas chromatography was performed with a Varian-3400 series unit (Hewlett-Packard, Palo Alto, California) equipped with a Finnigan MAT-90 (Finnigan MAT GmbH, Bremen, Germany). PCDDs, PCDFs, and coplanar PCBs were analyzed by the selected ion monitoring method. The column used was a DB-dioxin fused silica capillary column, 0.25 mm i.d.Å~60 m, with 0.25 mm film thickness (J&W Scientific, Folsom, California). Further details are described in the previous paper[1].

The toxicity of the dioxins was calculated by the WHO TEF method (1997) and is expressed as TEQ/g lipid. Statistical analysis. SPSS version 10 was used for the statistical analyses. Correlation analysis was performed between PCDD/PCDF/Co-PCB and various variables. Linear regression analysis and logistic analysis were used for evaluating the effects of dioxins, if a significant correlation ($p<0.05$) was obtained, and Odds Ratio was calculated if necessary.

Results

Characteristics of the workers:

The average age of the residents A (around incinerator) was 41.6+/-5.5 years old, and the average age of the female subjects was 40.4+/-4.9. The physical characteristics of the subjects were as follows: height; 169.2+/-6.0 cm in the males and 157.1+/-4.8 cm in the females; body weight, 68.8+/-9.5 Kg in the males and 54.0+/-6.9 Kg in females, body mass index, 24.0+/-3.1 in the males and 21.9+/-2.8 in the females; body mass index, 24.0+/-3.1% in the males and 21.9+/-2.8% in the females; systolic blood pressure, 126.8+/-14.1 mmHg in the males and 116.2+/-13.7 mmHg in the females; and diastolic blood pressure, 81.0+/-10.8 mmHg in the males and 70.9+/-9.1 mmHg in the females.

Blood dioxin levels:

The average concentrations of blood PCDD/PCDF/Co-PCB congeners are shown in Table 1. Congener-specific distribution is quite similar in 8 regions. TEQ of area A and control area B were as follows: PCDD; 8.1+/-3.4 pg TEQ/g lipid (2.9-18.8 pg TEQ/g lipid) vs. 9.5+/-5.6 pg TEQ/g lipid (2.5-26.6 pg TEQ/g lipid), respectively, PCDF; 5.4+/-3.7 pg TEQ/g lipid (1.5-23.1 pg TEQ/g lipid) vs. 6.3+/-4.0 pg TEQ/g lipid (1.2-27.6 pg TEQ/g lipid), respectively, co-PCB; 9.5+/-7.2 pg TEQ/g lipid (1.9-42.4 pg TEQ/g lipid) vs 5.8+/-4.3 pg TEQ/g lipid (0.6-20.6 pg TEQ/g lipid), respectively. Total TEQ was 23.0+/-10.7 pg TEQ/g lipid (8.7-66.5 pg TEQ/g lipid) in A area vs. 21.7+/-10.4 pg TEQ/g lipid (4.7-47.5 pg TEQ/g lipid) in control area.

Occupation and dioxin levels:

The concentration of PCDD/PCDF and Co-PCB by age showed a linear increase. When number of subjects in quartile concentration of PCDD, PCDF, and co-PCB was compared, farmers showed increasing trend in PCDD quartile, while clerks showed increasing trend in PCB (Table 2).

Dioxin levels and laboratory data:

Peripheral blood did not show any significant association with dioxin levels. Blood

biochemistry data are shown in Table 3.

Table 1. Blood dioxin levels of the residents (pg/g lipid)

	Control (n=81)			A area (n=95)		
Å@	mean	sd	median	mean	sd	median
D2378	0.9	0.4	0.8	0.9	0.7	0.5
D12378	5.0	3.7	5.0	4.9	4.2	4.2
D123478	2.4	1.9	2.0	1.9	1.4	1.6
D123678	44.8	33.8	35.3	33.8	16.6	30.3
D123789	6.3	5.6	4.1	4.2	3.4	3.3
D1234678	22.8	14.2	18.9	20.8	10.4	19.9
OCDD	514.8	644.5	281.0	474.9	528.5	333.3
F2378	0.7	0.4	0.6	0.7	0.5	0.5
F12378	0.8	0.5	0.5	0.8	0.7	0.5
F23478	9.9	7.1	8.6	7.5	7.4	6.3
F123478	4.2	2.5	3.8	5.8	3.9	4.3
F123678	5.0	3.1	4.5	4.9	2.3	4.4
F123789	0.5	0.1	0.5	1.3	1.6	0.5
F234678	2.7	2.2	2.3	3.5	2.6	2.7
F1234678	4.4	3.8	3.5	5.3	3.7	4.6
F1234789	0.5	0.2	0.5	0.7	0.8	0.5
OCDF	3.9	1.9	4.3	3.4	2.0	4.6
PCB77	11.4	7.8	10.6	21.7	20.4	13.5
PCB126	52.5	41.4	41.7	86.6	66.9	60.7
PCB169	59.0	42.4	50.0	79.3	71.0	55.2
D_TEQ	9.5	5.6	8.0	8.1	3.4	7.4
F_TEQ	6.3	4.0	5.6	5.5	3.7	4.3
DF_TEQ	15.8	8.4	13.4	13.5	6.0	12.0
PCB_TEQ	5.8	4.3	4.6	9.5	7.2	6.8
total_teq	21.7	10.4	20.7	23.0	10.7	21.0

Table 2. Occupation and subject number in each quartile blood concentration

	PCDD-TEQ				PCB-TEQ			
PCDD-TEQ	<5.2pg	5.2-7.5	7.5-10.6	10.6<pg	<3.4pg	3.4-5.8	5.8-10.2	10.2<pg
Agriculture (rice)	3	5	4	14	9	5	6	6
(vegetables)	2	5	6	10	3	7	9	4
Clerk	21	24	23	18	21	26	32	32
Housewife	8	8	8	7	6	9	7	9

Life style and dioxin levels:

The effects of dietary habits were analyzed by chief-component analysis. In food frequency, 68.4% of food intake was explained by 9 factors. Correlation analysis between these factors

and dioxin levels showed that factor 1 (fishcake, mayonnaise, ham, egg, margarine) was positively associated with PCDD TEQ, while factor 7 (milk and fermented soybean, natto) was negatively associated with PCDD/F levels. Food frequency questionnaire was repeatedly observed by frequency of ordinary Japanese meal intake. Factor 5 (vegetables and fried dishes) was significantly associated with PCDD/F levels. Frequency to eat various fishes related to the blood dioxin levels.

Table 3. Selected laboratory data by area

	Control (n=81)		Area A (n=95)	
Å@	mean	sd	mean	sd
gamma-GTP	38.6	51.5	45.0	62.3
Glucose	90.6	11.5	96.8	16.8
Total protein	7.9	0.6	8.2	0.4
Triacylglyrecol	108.8	69.4	134.9	95.4
Total cholesterol	187.8	34.1	195.2	31.6
HDL-Cholesterol	58.7	19.4	56.7	19.3
CD3 (pan T)	71.6	7.8	70.4	6.4
CD4 (helper T)	42.2	10.6	43.9	7.5
CD8 (suppressor T)	29.2	5.7	27.7	7.6
CD4_8 ratio	1.5	0.6	1.8	0.8
CD56 (NK marker)	14.8	7.3	16.8	6.9
NK activity	24.3	15.8	29.0	13.7

Past and present history of diseases:

Most subjects appeared healthy but reported various diseases during the last 10 years in their past history of diseases: hypertension in 10, diabetes mellitus in 3, hyperlipidemia in 10, allergy in 26, atopy in 20, peptic ulcer in 20, and others. No past history was in 100. Odds ratio by PCDD/PCDF dichotomous levels was 3.17 in hypertension, 2.02 in diabetes mellitus, 2.0 in hyperlipidemia, 0.70 in allergy, 0.75 in peptic ulcer.

Discussion

Workers in one municipal incinerator showed accumulation of PCDD/PCDF/Co-PCB in blood lipid, ranging from 7.0 pg TEQ/g lipid to 831.19 pg TEQ/g lipid. Some residents who showed increased blood PCDF seemed to be influenced by the incinerator. In Japan, 90% of the daily intake of PCDDs, PCDFs, and other dioxin-like compounds is estimated to come from food. The difference in body burden between workers and residents outside the incinerator must enter the body through the lungs and skin. In general, residents around incinerator did not show different dioxin levels from those in the control area.

A history of hypertension and hyperlipidemia had increased odds ratios. The history was self-reported, so confirmation of the diagnosis may be necessary. Prediction of future health effects in the subjects with low-level exposure is difficult. Very long half-life of dioxins inside the body, however, may cause various effects at the lipid membrane of lipoprotein and cells. Follow-up of the chronically exposed people should be important.

References

1. Kitamura K, Kikuchi K, Sunaga K, Watanabe S, Iida T, Waechter G, Takada T. Health effects by dioxin exposure in the municipal incinerator. J Epidemiol (in press).
2. Watanabe S, Kitamura K, Nagahashi N. Effects of dioxins on human health. J Epidemiol 1-13 (1999)