DETECTION OF THE LUNG CANCER CAUSING SUBSTANCES IN THE ATOMOSPHERE – DETECTION OF THE ENVIRONMENTAL POLLUTANTS IN TISSUES OF THE DOG

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Introduction

In Japan, the cerebral apoplexy brought the highest death rate in a statistics of death cause during a period of after World War II to the middle of 1950's. Thereafter, however, the death toll by malignant neoplasm increased remarkably year by year, and then the death rate became the highest by 1970's. Even in recent years, the death rate has been remarkably increasing. Especially, the lung cancer gave a higher increasing death rate than did other tumors. Consequently, in 1999, the lung cancer occupied 21.6% of the all tumor death toll in the men and 12.4% in the women. Therefore, the detection of causal factors was one of the most important subjects. Similar phenomenon was seen in Taiwan, Republic of China.

Contrast in a striking way with other organs, the lung is directly attacked by pollutants in the atmosphere through breath. Especially, pollutants in the suspend particle are speculated to accumulate and give adverse effects during a long time. Taking these facts into consideration, atmospheric pollutants are strongly considered to be the primary causal agent for the rapid increase of lung cancer.

It is well known that the attack of malignant neoplasm has a strong relation with the life style, especially such as smoking and drinking. Therefore, we selected the most suitable experimental animal for detection of lung cancer causing pollutants in the atmosphere, because the dog live in the same environment as do human beings, has a bigger breath volume per weight than human beings and has no habits of smoking and drinking. In this study, we obtained three kinds of lung, liver and adipose tissue from dogs in Japan and Taiwan, analyzed for many atmospheric pollutants in order to detect lung cancer causing agents.

Materials and Methods

1) Sample:

Samples of lung, liver and adipose tissue were obtained from 3 male dogs (Age: 5 - 7 years, body weight: 13.5 – 23.0 kg) and 4 female ones (Age: 3 - 7 years, body weight: 11.0 – 31.5 kg) in June, 2000 in Taichung Prefecture, Taiwan, and from 4 male dogs (Age: 2 - 8 years, body weight: 9.0 – 25.0 kg) and 5 female ones (Age: 2 - 6 years, body weight: 9.0 – 21.0 kg) in September to November, 2001 in Kagoshima Prefecture, Japan. Taichung Pref. is located at middle Taiwan and has a population of 1,514,000. On the other hand, Kagoshima Pref. is located at southern Japan and has a population of 1,770,000.

Three air samples were obtained during a period of 5 to 26 November 2001 in Kagoshima City using a high volume air sampler with a glass fiber filter (GFF) for collection of particle phase pollutions and two polyurethane form plugs (PFU) for vapor phase ones.

2) Analytical method:

The sample of lung (200 g), liver (200 g) or adipose tissue (20 g) was mixed with anhydrous Na_2SO_4 for dryness and extracted with 50% dichloromethane/n-hexane (400 ml for lung and liver, and 100 ml for adipose) for 4 hrs. under reflux. After filtration, the extract was divided into three portions for the measurements of lipid content, dioxin analogues and organochlorinated pesticides/poly aromatic hydrocarbons, respectively.

2-1) Analytical method for dioxins (PCDDs, PCDFs and Co-PCBs) :

A portion of the extract was concentrated, added with $^{13}C_{12}$-labeled internal standards and decomposed with 1 M KOH/ethanol for 2 hrs under shaking at room temperature. After addition of water, the treated extract was extracted twice with n-hexane, washed and dried over anhydrous Na_2SO_4. The hexane extract was cleaned up according to our previous method[1.] The cleaned up extract was analyzed in EI-SIM mode at a resolution of 10,000 using a Hewlett Packard 5890J GC-JEOL M700 MS.

2-2) Analytical method for organochlorinated pesticides/poly aromatic hydrocarbons (PAHs):

A portion of the extract was concentrated, dried, dissolved with 30% acetone/cyclohexane and cleaned up by gel permeation column (Showa Denko K. K., Japan, CLNpak EV-2000, 4 mm x 300 mm) chromatography. The elute portion of 70 – 300 ml was sampled, concentrated and cleaned up on a silica gel column (Merck, Kieselgel 60, 60 – 230 mesh, 1.5 g) with an elution of 5% diethyl ether/n-hexane. The elute was concentrated, dried, dissolved with n-decane and analyzed for organochlorinated pesticides **(**HCHs, DDTs, Chlordenes and HCB) and PAHs in EI-SIM mode at a resolution of 10,000 using a Hewlett Packard 5890J GC-JEOL M700 MS.

2-3) Analytical method for air sample:

The air sample was cleaned up for pollutants.

According to our previous method[1),] the GFF and two PUFs were respectively cut into small pieces and were extracted with toluene and acetone. The two extracts were respectively divided into two portions and analyzed for dioxin analogues and organochlorinated pesticides/PAHs, respectively, basically according to above described methods.

Results and Discussion

1) Dioxins

As shown in Table 1, there was observed a great difference in the pollution level of dioxins in the organ/tissue samples (0.67–62 pgTEQ/g-lipid) among dogs in Kagoshima Prefecture, Japan. The average level was arranged in order of liver (18 pgTEQ/g-lipid) >> lung (4.4 pgTEQ/g-lipid) > adipose tissue (2.6 pgTEQ/g-lipid), suggesting dioxins to bind covalently with the liver cell protein. On the other hand, the similar observation was also recognized in the case of dogs in Taichung Prefecture, Taiwan, showing the organ/tissue pollution level to be in a wide range of 1.6 – 48 pgTEQ/g-lipid. However, compared to the case of Japan, the average accumulation level was somewhat higher.

2) Organochlorinated pesticides

HCHs (α–, β–, γ– and δ–HCHs), Drins (Aldrin, Endrin and Dieldrin), Chlordans (Heptachlor, Heptachlor Epoxide, *cis*-Chlordan, *trans*-Chlordan, Oxychlordan and Nonachlor) and Hexachlorobenzene (HCB) were surveyed for this study. As well as the case of dioxins, a great

Table 1. Accumulation levels of Dioxin analogues and organochlorinated pesticides in

dogs's tissues in Kagoshima Prefecture, Japan and Taichung Prefecture, Taiwan

Compound	Kagoshima, Japan			Taichung, Taiwan		
	Lung	Liver	Adipose	Lung	Liver	Adipose
Dioxin analogues*	4.4	18	2.6	15	31	6.7
	(1.9–11)	(3.1–62)	(0.67–5.9)	(7.8–36)	(8.5–48)	(1.6–24)
Total HCHs**	11	22	30	7.0	7.0	5.1
	(ND–77)	(0.32–97)	(0.66–230)	(ND–16)	(1.3–16)	(ND–11)
Total DDTs**	2.1	0.55	0.64	0.011	3.0	0.34
	(ND–7.7)	(ND–97)	(0.66–230)	(ND–6.8)	(0.31–7.9)	(ND–11)
Total Drins**	11	5.5	19	62	37	0.49
	(ND–34)	(0.46–20)	(ND–140)	(0.51–300)	(0.14–140)	(0.29–0.85)
Total Chlordans**	29	5.3	5.2	16	25	13
	(2.3–66)	(1.3–13)	(1.6–18)	(5.6–33)	(2.1–32)	(0.7–17)
HCB**	1.1	0.17	0.14	0.59	0.80	0.63
	(2.3–66)	(1.3–13)	(1.6–18)	(0.10–0.91)	(0.043–0.94)	(0.10–0.86)

*: pgTEQ/g-lipid **: ng/g-lipid ND: Not detected
Figures in parenthesis show a range of the minimum to maximum.

variance in the pollution level of each compound was observed among dogs in Kagoshima (Table 1). Chlordans were detected at a higher frequency in comparison with other chlorinated pesticides. The accumulation level was remarkably higher in the lung than the liver and adipose tissues, suggesting the main pollution source to be air. The similar tendency was observed in the cases of DDTs and HCB. Compared to Japanese dogs, Taiwanese ones showed higher levels of Drins in the lung and liver with the levels of ca. 6 times and 7 times greater, respectively.

In Japan, the level of total chlordans was remarkably higher in the lung (29 ng/g-lipid) than the liver (5.3 ng/g-lipid) and adipose tissue (5.2 ng/g-lipid). However, in Taiwan, the highest level was seen in the liver (see Tabel 1).

As described above, there were some discrepancies in the accumulation pattern of chlorinated pesticides between Japanese and Taiwanese dogs, suggesting their respective distribution pattern in the environment to be different in the both countries.

3) PAHs

Twelve kinds of PAHs were analyzed in this study. In dogs in Kagoshima, low toxic and carcinogenic PAHs with three aromatic rings such as phenanthrene and anthrathene occupied most of the total PAH level. In this study, however, we focused on high toxic and carcinogenic PAHs with more than four aromatic rings, in order to determine a causal agent for lung cancer. These toxic PAHs were found to accumulate strikingly in the lung than other organs in six of nine dogs, showing the average level to be 1,200 ng/g-lipid in the lung, 600 ng/g-lipid in the liver, and 55 ng/g-lipid in the adipose tissue.

Similar results were also observed in six of seven dogs in Taiwan. The average levels in the lung, liver and adipose tissue were respectively 1,100, 130 and 17 ng/g-lipid. In addition, more toxic PAHs with five and six rings were relatively larger in the lung than other organs from dogs in both

Table 2. Average benzo(a)pyrene toxicity equivalency quantity of PAHs with 4–6 aromatic rings in

dogs's tissues in Kagoshima Prefecture, Japan and Taichung Prefecture, Taiwan

Compound	Kagoshima, Japan			Taichung, Taiwan		
	Lung	Liver	Adipose	Lung	Liver	Adipose
Fluoranthene	0.57	0.77	0.024	0.16	0.047	0.078
Pyrene	0.44	0.59	0.025	0.11	0.030	0.089
Benzo(*a*)anthracene	2.1	1.9	0.047	1.3	0.90	1.4
Chrysene	0.35	0.34	0.0076	0.26	0.082	0.11
Benzo(*b*)fluoranthene	1.6	0.99	0.030	30	1.8	1.2
Benzo(*k*)fluoranthene	0.66	0.83	0.011	8.4	0.12	0.28
Benzo(*a*)pyrene	4.3	4.1	0.10	7.5	0.80	1.2
Indeno(*1,2,3-cd*)pyrene	0.37	0.07	0.0023	17	0.10	0.027
Benzo(*ghi*)perylene	0.027	0.0074	0.00076	1.0	0.013	0.074
Dibenzo(*ah*)anthracene	1.5	N.D.	N.D.	42	0.15	0.000
Total	12	9.7	0.25	108	4.0	4.4
Relative concentration*	60	49	1.0	25	0.91	1.0

* : The concentration in the adipose tissue = 1.0

of Kagoshima and Taiwan.

Benzo(a)pyrene toxicity equivalency quantity ($TEQ_{B(a)P}$) was calculated for PAHs with more than four rings on the basis of a proposal factor by Nisbete and LaGoy (1992)[2]. As shown in Table 2, the average $TEQ_{B(a)P}$ were arranged in order of the lung (12 ng-$TEQ_{B(a)P}$ /g-lipid) > the liver (9.7 ng-$TEQ_{B(a)P}$ /g-lipid) >> the adipose tissue (0.25 ng-$TEQ_{B(a)P}$ /g-lipid) in nine dogs in Kagoshima, and in order of the lung (108 ng-$TEQ_{B(a)P}$/g-lipid) > the liver (4.0 ng-$TEQ_{B(a)P}$/g-lipid) , the adipose tissue (4.4 ng-$TEQ_{B(a)P}$/g-lipid) in seven dogs in Taiwan.

Although there was a considerable variation in the constituent of PAHs among three air samples obtained in Kagoshima, the majors were PAHs with three and four aromatic rings in the vapor phase, and were PAHs with more than five aromatic rings in the particle phase. Taking these results into consideration, the high toxic and carcinogenic PAHs in the lung might be mainly derived from the particle phase in the air.

Conclusion

1) The accumulation level of Dioxins was arranged in order of liver > lung > adipose tissue in the both cases of Kagoshima, Japan and Taichung, Taiwan.
2) Total Chlordans, HCB and toxic PAHs were accumulated more abundantly in the lung than the liver and adipose tissue of the dogs from Kagoshima Prefecture, Japan.
3) Total Drins and toxic PAHs were accumulated more abundantly in the lung than the liver and adipose tissue of the dogs from Taichung Prefecture, Taiwan.

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