Promoter and Mutagenic Activity of Particulate Matter Collected from Urban Air

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Urban air particulate samples were collected on a quartz fiber filter with a High Volume Air Sampler, which was placed on the roof of the National Institute of Public Health, Minato-ku, Tokyo. Those samples were tested in both transformation assay using Bhas42 cells (Bhas assay) as a bioassay for cancer promoters and in the Ames microsuspension method as for initiators. Good dose–response relations were observed with both testing methods, and the samples showed mutagenicity and promoter activity. A comparison per particle weight between the samples collected in spring and autumn indicated that the value was higher in autumn samples, suggesting that mutagenic substances and cancer promoters might be polluting the air and be correlated with each other. Therefore, to monitor the carcinogenic-related activity of air pollutants, not only the mutagenicity assay but also bioassays for cancer promoters are required.

Key words —— airborne particulate matter, transformation assay, microsuspension, Bhas42 cell, promoter activity

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INTRODUCTION

Various hazardous substances are present as gases or particles in urban air. Those particulate substances contain trace amounts of carcinogens with relatively high molecular weights, such as benzo[*a*]pyrene, which is a carcinogen and suspected to be an exogenous endocrine disruptor. Exposure of the respiratory tract to such substances may cause health effects,[1–3] and thus it is important to assess the existence of these substances and exposure to them to take measures to prevent cancer. Mutagenicity testing has gradually been applied as a bioassay to assess the initiator activity of trace amounts of environmental pollutants and is now being considered as a method for monitoring airborne particulate matter.[4,5] However, little is known about cancer promoters because *in vitro* bioassays for them have not been widely used. Therefore we measured both the activity of airborne particulate matter collected in the Tokyo metropolitan area in the transformation assay using Bhas42 cells (Bhas assay) and the microsuspension method, which is an improved process of the Ames method, and the results were compared.

MATERIALS AND METHODS

Reagents and Media ——

Solvents: Pesticide residue analysis-grade dichloromethane (DCM; Kokusan Chemical Works Ltd., Japan) and fluorometric analysis-grade dimethylsulfoxide (DMSO; Dojin Chemical Laboratory Ltd., Japan) were used. Metabolic activation system: S9/Cofactor A set for the Ames test (S9 mix; Oriental Yeast Co. Ltd., Japan) was used. The DME/F12 medium was supplemented with 5% fetal calf serum (FCS) for transformation experiments.

Sampling of Airborne Particles —— On the National Institute of Public Health building located in the center of Tokyo, (Shirokanedai, Minato-ku, Tokyo) Japan, airborne particulate samples from ambient air were collected in a quartz fiber filter (20 × 25 cm, Pallflex Products Co., U.S.A.) at a flow rate of about 1.3 m^3/min using a High Volume Air Sampler (Kimoto Electric Co., Japan). The filter samples were stored in a deep-freeze (–80°C). Then they were divided according to the season into spring (March, April, and May), summer (June, July, and August), autumn (September, October, and November) and winter (December, January, and February).[6] These

Table 1. Samples Used for this Study

Sample no.	Sampling period	Air volume (m^3)	Airborne particle (mg)
I 1980 spring	(3 Mar–27 May)	1360.5	112.8
II 1981 spring	(3 Mar–27 May)	1407.7	122.3
III 1983 spring	(1 Mar–31 May)	1783.9	130.9
IV 1980 autumn	(5 Sep–29 Nov)	1026.8	154.1
V 1981 autumn	(5 Sep–29 Nov)	1549.3	136.2
VI 1983 autumn	(3 Sep–27 Nov)	1432.7	122.0
Average		1426.8	129.7

filter samples were collected and stored every 6 days so that each group included approximately 15 samples. In this study, 1980 spring, 1980 autumn, 1981 spring, 1981 autumn, 1983 spring, and 1983 autumn samples were used (Table 1).

Extraction of Organic Substances —— Each filter was cut into small pieces and put into a centrifuge tube. Ten milliliters of DCM was added, and the samples were extracted by sonication for 10 min. After filtration, the solution was evaporated under a mild nitrogen stream. The organic extracts were stored at –80°C until tested.

Transformation Assay[7] —— Bhas42 cells (4×10^4 cells), which were established from BALB/c 3T3 cells transfected with the v-Ha-*ras* oncogene, were seeded into 6-well plates. Three, 7, and 10 days later the medium was changed, which contained various concentrations of samples. On day 14, the medium was changed to DME/F12. On day 17, the cells were fixed with methanol and visualized by Giemsa staining to measure the numbers of transformation foci. 12-*O*-Tetra-decanoyl-phorbol-13-acetate (TPA) was used as a positive control.

Mutagenicity Test —— The mutagenicity test was conducted using a microsuspension procedure that was a slight modification of Kado *et al.*'s method[8] using *Salmonella typhimurium* TA100 and TA98 strain[9] ($\times$ 20 conc. bacterial solution) both with and without a metabolic activation system (S9 mix).

RESULTS AND DISCUSSION

Initiation and promotion have long been regarded as important factors in the mechanism of carcinogenesis.[1] Chemical substances related to the initiation process where genes initially mutate can be detected easily with the mutagenicity testing method using bacteria. However, since cancer promoters cannot be detected with bacterial systems, the use of cultured cells has been investigated. Cells that have already initiated have recently been developed, and the Bhas assay that can relatively easily detect cancer promoter activity has also been developed and is now being validated. In this research, we used this method as well as the microsuspension method.

Figure 1 shows measurements of airborne particulate matter obtained with the Bhas assay. The dose in Fig. 1 is the value converted into particulate weight. A good dose–response relation was found between particulate weight and the number of foci, and cytotoxicity may appear in the high-concentration area (200 μg/well). It was also observed that the positive control TPA (20 ng/ml) gave numerous foci (21.0 ± 3.10, 27.2 ± 3.87), which is almost four-fold that of the negative control (4.17 ± 2.32, 6.67 ± 2.58).

Figure 2 shows measurements of the same airborne particulate samples obtained using the microsuspension method. All 6 samples indicate a clear dose–response relation irrespective of whether strain and S9mix were added or not. In strain TA98, mutagenicity decreased significantly when S9mix was added compared with that when it was not added. At the same time, in strain TA100, there was almost no difference.

Promoter activity and mutagenic activity (when S9mix was not added) per particulate volume were then derived from the least-squares method of the linear part of the dose–response relation curves in Figs. 1 and 2. Table 2 shows the results. A slight difference is observed by year of collection, but there is clearly a significant difference in both activities between spring and autumn. These findings indicate that not only a mutagenicity test for cancer initiators but also a bioassay for cancer promoters is essential for monitoring the carcinogenicity of airborne particulate matter. To elucidate the cause of the higher activity of both initiators and promoters in

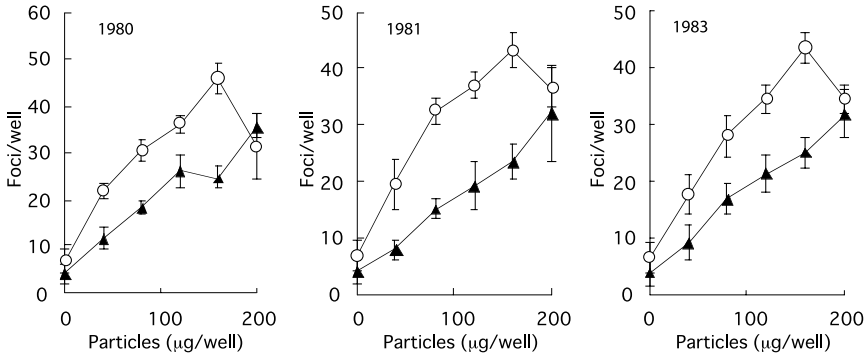

Fig. 1. Results of Bhas Assay with Airborne Particulate Samples
—○— Autumn, —▲— Spring.

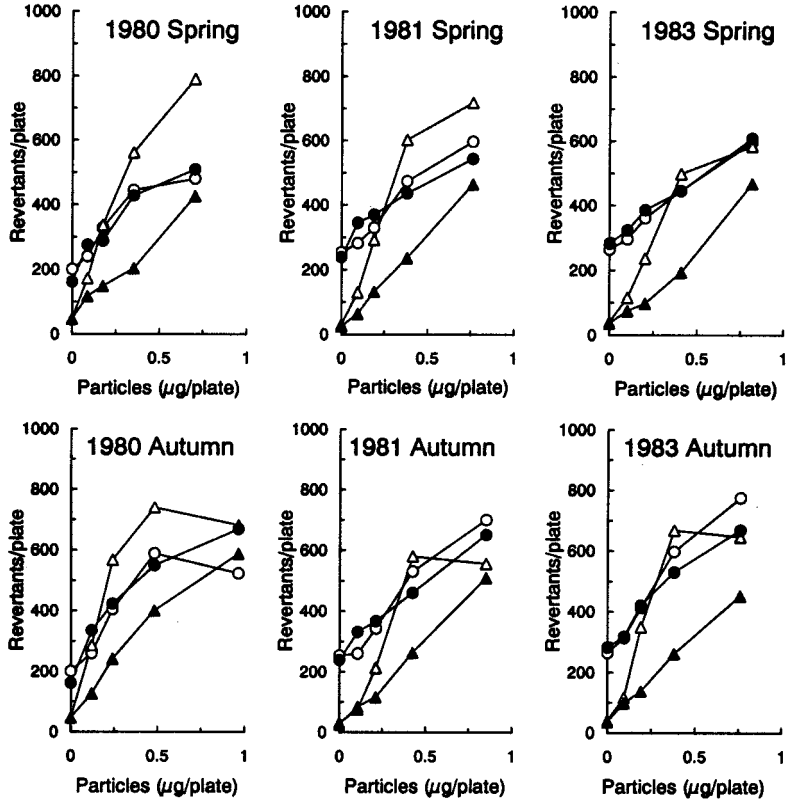

Fig. 2. Results of Microsuspension Test with Airborne Particulate Samples
—○— TA100 (–S9mix), —△— TA98 (–S9mix), —●— TA100 (+S9mix), —▲— TA98 (+S9mix).

Table 2. Promoter and Mutagenic Activity of Airborne Particles

Sample	Promoter activity (foci/mg)	Mutagenic activity (rev./mg)	
		TA100 (−S9)	TA98 (−S9)
1980 spring	182	368	1004
1981 spring	137	500	883
1983 spring	136	480	2274
1980 autumn	232	706	1391
1981 autumn	227	592	1175
1983 autumn	226	815	3410

autumn than in spring, further investigation and measurement of other components and their analysis are required.

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