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Monitoring of the Distribution of Ambient Air Particles in Seoul Using a Cascade Impactor and the Particle Toxicity

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ABSTRACT

The distribution of ambient air particles varies according to climate, industries, and other sources. In this study, ambient air particles (less than 12.1 μm) were monitored from February to August, 2007 as 12 different fractions sorted by a cascade impactor. Particles in the size range from 0.33 μm to 0.76 μm comprised the main fraction of ambient air particles in Seoul, Korea. On the day of an Asian dust event, the particle fraction size increased to 1.25～2.5 μm. The different sized particle fractions were also monitored for metals and were found to contain toxic heavy metals including Pb, Cd, Hg, Cr and As. Particle preparations were significantly cytotoxic when exposed to cultured BEAS-2B cells. Microarray analysis of the treated cells indicated a significant up-regulation of a number of genes associated with oxidative stress, including metallothionein, heme oxygenase-1, heat shock protein 70, and NAD(P)H dehydrogenase-1.

Key words : Air particles, Monitoring, Toxicity, Gene expression

INTRODUCTION

The concentration of particulate matter (PM) in ambient air is known to adversely affect human health (Roberts and Martin, 2007; Yang *et al.*, 2009). Recent epidemiological studies have reported a close association between PM levels and increased hospital admissions for respiratory and cardiovascular diseases (Wilson *et al.*, 2004; Chang *et al.*, 2005; Hart *et al.*, 2006). Therefore, many countries have now established air quality standards for specific class sizes of PM, notably PM_{10} and $PM_{2.5}$. Recently, ambient nanoparticles have emerged as health concerns due to their growing contribution to ambient PM. In contrast to engineered nanoparticles, ambient nanoparticles are generated by natural processes and by human anthropogenic activity.

One of the primary sources of environmental nanoparticles is the consumption of fossil fuels. With the development of industry and technology, the consumption of fossil fuel continues to steadily increase. The combustion of fossil fuels release heavy metals, PAHs (polyaromatic hydrocarbons), and gas phase components as well as nanoparticles into the air (Lewtas, 2007; Naspinski *et al.*, 2008; Liu *et al.*, 2008). Toxicologists have suggested that when nanoparticles reach the alveolar regions of the lung, they remain in lung tissue for prolonged times and cause serious unfavorable health effects (Oberdörster *et al.*, 2005; Stone *et al.*, 2007; Valavanidis *et al.*, 2008).

However, the actual distribution of nanoparticles in

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the ambient air particulate matter has yet to be investigated. In a previous study, we reported that the level of TSP (total suspended particles) and PM_{10} in the ambient air showed seasonal variations, while $PM_{2.5}$ remained at an almost constant level throughout the year (Park *et al.*, 2008). We did not categorize the size distribution of the ambient nanoparticles in this seasonal study.

Therefore, in the present study, we collected ambient particles (less than 12.1 μm) classed into 13 different fractions by particles size, from February to August, 2007. We also tested the collected particles for toxicity towards cultured a human bronchial epithelial BEAS-2B cell line and analyzed particle exposure effects on expression of genes related to oxidative stress.

MATERIALS AND METHODS

1. Sampling

Sampling was performed on the roof of a university building at a height of 12 meters above the ground from February to August, 2007. Size distribution of the ambient particles was determined using an ambient cascade impactor (Dylec Inc., Model No. LP-20) with a built-in Teflon filter (Model No. T60A20, Dylec Inc., Japan, Tokyo). This sampler divided total ambient particles into 12 fractions according to their particle size (size; <0.06, 0.06～0.13, 0.13～0.22, 0.22～0.33, 0.33～0.52, 0.52～0.76, 0.76～1.25, 1.25～2.5, 2.5～3.9, 3.9～5.7, 5.7～8.5, and 8.5～12.1 μm).

2. Metal analysis in the particle preparations

To determine the heavy metal concentrations in the particle fractions, four fractions were re-categorized by pooling the materials collected on the membrane filters: <0.06 μm, 0.06～0.52 μm, 0.52～3.9 μm, and 3.9～12.1 μm. For metal analysis, filters were extracted and digested in a mixture of 70% HNO_3 (7 mL) and 30% H_2O_2 (1 mL) using a microwave digestion system (Milestone, Sorisole, Italy). The concentration of each metal was analyzed by ICP-MS (Elan 6100/Perkin Elmer, USA) by the Korean Basic Science Institute (KBSI, Seoul, Korea). The concentration of metals was calculated as ng/m^3 ambient air. Particle preparations for toxicity studies were prepared by extracting the membrane filters with water. The water extract was lyophilized and re-suspended in culture media at the desired concentration for the cytotoxicity test.

3. Cell culture and cell viability test

BEAS-2B cells were maintained in Dulbecco's modified Eagle's medium (DMEM)/F12 (GIBCO Invitrogen, Seoul, Korea) containing 10% FBS, penicillin 100 IU/mL, and streptomycin 100 μg/mL. Cells were grown and maintained in 28 cm^2 cell culture flasks at 37°C in a 5% CO_2 humidified incubator. Cell viability was measured by the MTT assay. Briefly, cells were seeded on 96-well tissue culture plates at 3×10^3～1×10^4 cells per well. After a 24 hour stabilization period, the cells were treated with particle preparations for 24, 48, 72, or 96 hours. At the end of the exposure, MTT solution (Sigma-Aldrich, St. Louis, MO, USA) was added, and the cells were incubated for an additional 4 hours at 37°C. After solubilization with DMSO, absorbance of the formazan product was measured at 540 nm using a microplate reader (VersaMax, Molecular Devices, Sunnyvale, CA, USA). The results are presented as the mean$\pm$S.D. of 4 separate experiments. The viability of the treated group was expressed as a percentage of that of the control group, which was assumed to be 100%.

4. Microarray

For the analysis of the gene expression profiles, cells were treated for 12 hours with a particle preparation at 2 mg/mL, which was derived from material collected from July to August, 2007. Total RNA was prepared using the RNAgent Total RNA Isolation System (Promega, Madison, WI, USA). Micro-

array assays were carried out using Applied Biosystems Human Genome Survey Arrays. Briefly, digoxigenin-11-UTP labeled cRNA was generated and linearly amplified from 1 μg of total RNA purified from the control and particle-treated group, using an Applied Biosystems Chemiluminescent RT-IVT Labeling Kit. Array hybridization, chemiluminescence detection, image acquisition, and analysis were performed using an Applied Biosystems Chemiluminescence Detection Kit and an Applied Biosystems 1700 Chemiluminescent Microarray Analyzer following manufacturer's protocol. Each image was collected for each microarray using the 1700 Analyzer, which is equipped with a high-resolution, large-format CCD camera for gene expression analysis. Images were auto-gridded and the chemiluminescence signals were quantified, corrected for background and spots, and spatially normalized. Two experiments were performed separately.

RESULTS

1. Seasonal variations of particle distribution

To investigate seasonal variations of particle distribution, thirteen fractions were monitored using a cascade impactor for 23 weeks, from February to August, 2007. The particle size of each fraction has been described in Materials and Methods. Sampling was performed for 156 hours per week. In Fig. 1, fraction 8.5～12.1 μm (designated as ＜12.1 in Fig. 1) showed peak levels when it was collected during the Asian dust event, on 3/27～4/3 (month/day). The concentration of Fraction 12.1 during monitoring periods ranged from 20.11 $\mu g/m^3$ (7/10～7/16) to 166.35 $\mu g/m^3$ (3/27～4/3). During the period of the Asian dust event, fraction 1.25～2.5 μm (designated as 2.5) was the dominant fraction. In the spring season, the dominant fraction seemed to be the 0.33～0.52 μm fraction (designated as 0.52), but in the summer, the dominant fraction shifted to the 0.52～0.76 μm fraction (designated as 0.76). The nanoparticle fraction (size: less than 0.13 μm) was observed throughout the sampling period, although its level was never very high (Fig. 1).

2. Seasonal variations of metal concentrations

To investigate the seasonal variation of metal concentrations in the ambient air particles, four fractions were re-categorized by pooling the membrane filter contents: ＜0.06 μm, 0.06～0.52 μm, 0.52～3.9 μm, and 3.9～12.1 μm. Metal components were extracted from filters using a microwave system and analyzed by ICP-MS. As shown in Fig. 2, the concentration of Fe and Mn in Fraction 12.1 was proptional to the level of Asian dust (3/27～4/3). The peak concentrations of Fe and Mn were 91.97 ng/m^3 and 3489.11 ng/m^3, respectively. The concentrations of heavy metals in total ambient particles (less than 12.1 μm) during the sampling periods were as follows (the unit is ng/m^3, sampling day is written in parenthesis): Cr 13.19 (3/5～3/12)～168.9 (5/21～5/28), Mn: 22.38 (3/5～3/12)～91.97 (3/27～4/3), Fe: 1136.00 (2/26～3/4)～3489.11 (3/27～4/3), As: 1.75 (3/5～3/12)～17.02 (6/11～6/18), and Pb: 0.89 (5/4～5/11)～79.13 (6/11～6/18).

When total particles were divided into four fractions as shown in Fig. 3, the concentrations of Fe, Mn, and Pb increased as the particle size of the fraction increased. In contrast, the concentrations of As and Cr were detected at a similar level in all of the fractions.

3. Cytotoxicity of particle preparations

To investigate the cytotoxicity of particle preparations, two different preparations were prepared. One was obtained from TSP (total suspended particles) collected from March to April (called ADP), and the other was from TSP collected from July to August, 2007 (called NADP). During the sampling time of the ADP collection, Asian dust events occurred 5 times. The size of the ADP preparation was therefore larger than 1.25 μm, whereas the size of the NADP preparation was less than 0.76 μm. The concentrations of Cr, Pb, and Fe were higher in the ADP preparation than in the NADP preparation, while As

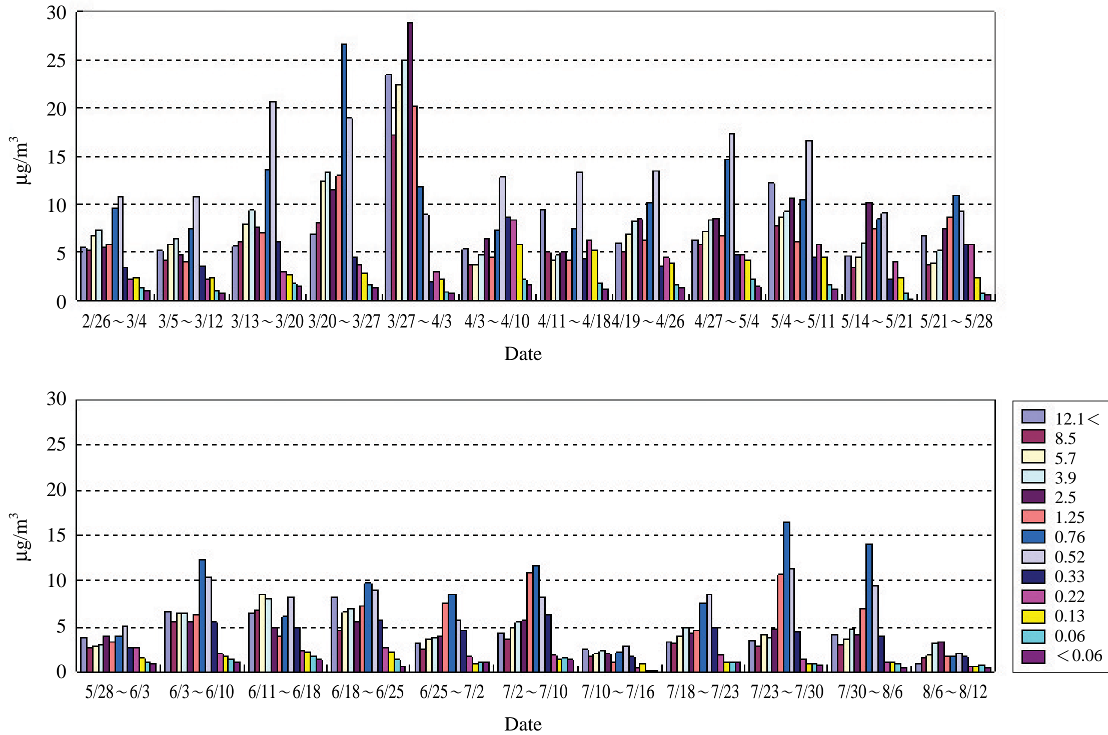

Fig. 1. Monitoring of 12 ambient air particle fractions collected in Seoul using a cascade impactor. Monitoring was performed from February 2007 to August 2007 on a weekly basis. The sampler divided total ambient particles into 12 fractions according to their particle sizes (size; <0.06, 0.06～0.13, 0.13～0.22, 0.22～0.33, 0.33～0.52, 0.52～0.76, 0.76～1.25, 1.25～2.5, 2.5～3.9, 3.9～5.7, 5.7～8.5, and 8.5～12.1 μm). Sampling time was designated in the figure. Each particle fraction was presented as the number of maximum size (12.1< means the fraction of 8.5～12.1 μm and 8.5 means the fraction of 5.7～8.5 μm, and so on).

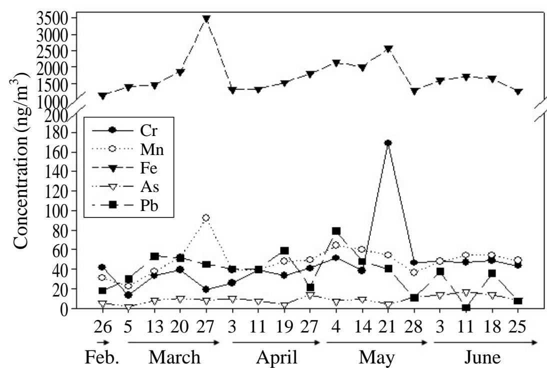

Fig. 2. Monitoring of metal concentrations in total ambient particles (less than 12.1 μm).

and Hg were higher in the NADP preparation.

As shown in Fig. 4, NADP extracts at 2 mg/mL showed a cytotoxicity of 72.3±1.5%, while ADP extracts showed cytotoxicity of 18.5±0.9% after 24 hours exposure of the cells. Both extracts decreased the viability of BEAS-2B cells in a time- and dose-dependent manner. Cell viability at 96 hours after treatment had decreased to 33.1±5.1% with the ADP preparation (Fig. 4A) and 11.3±0.7% with the NADP preparation (Fig. 4B).

4. Changes in gene expression induced by exposure to a particle preparation

Changes in gene expression induced by exposure to the NADP particle preparation were investigated. Cultured BEAS-2B cells were treated with 2 mg/mL NADP preparation for 12 hours. As shown in Table

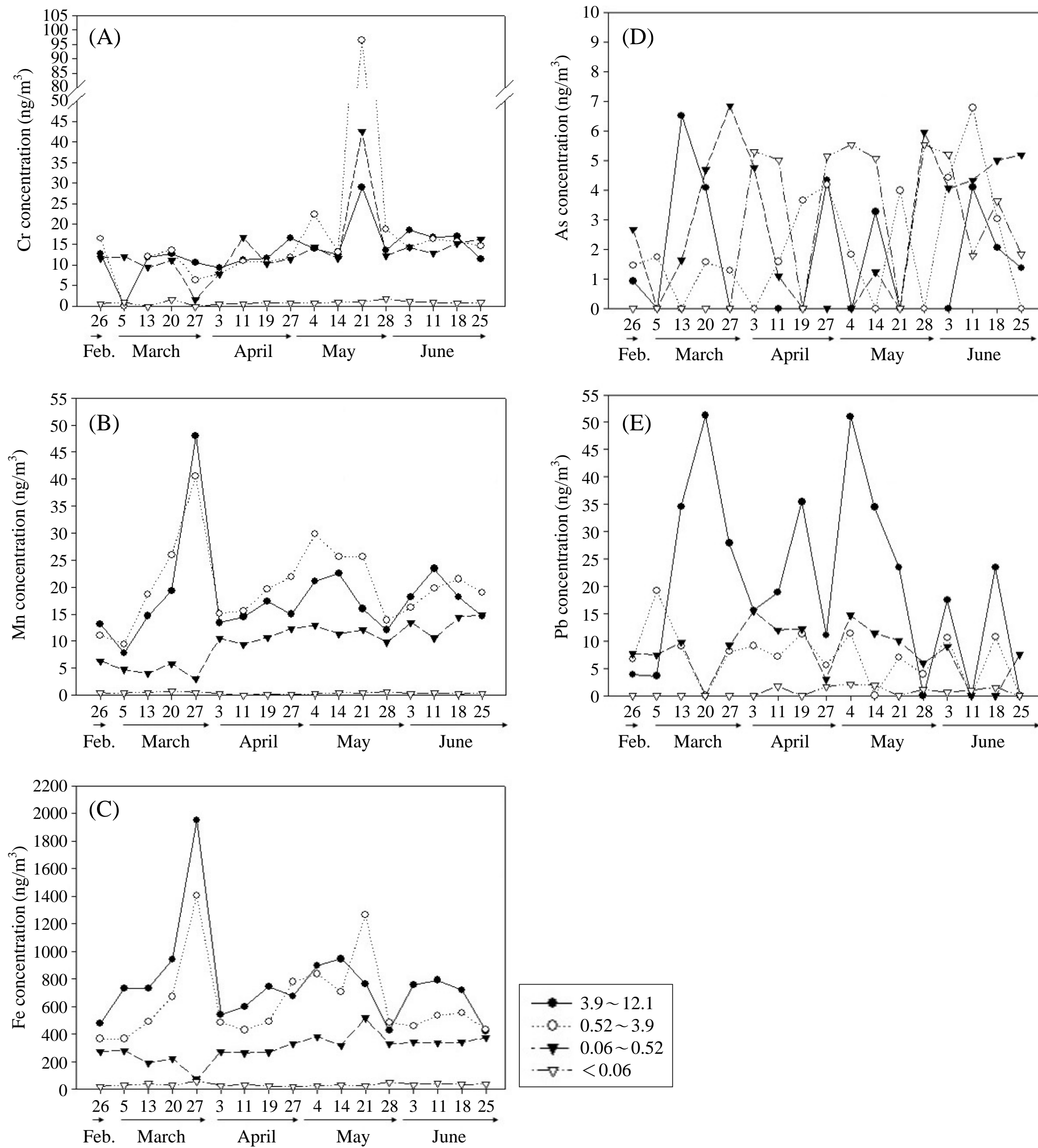

Fig. 3. Monitoring of metal concentrations in the four different particle fractions. Total particles were divided into four fractions as shown in the figures. Numbers in the insert mean the particle size. A: Chromium, B: Manganese, C: Iron, D: Arsenic, E: Lead.

1, the expression of 45 genes including metallothionein, heme oxygenase-1, MMP1 (matrix metalloproteinase-1), and IL-24 were up-regulated more than 2 fold. A total of 56 genes, including NPPB (natriuretic peptide precursor B), CMKOR1 (chemokine orphan receptor 1), OLR1 (oxidized low density lipoprotein (lectin-like) receptor), and GAS1 (growth arrest-specific), were down-regulated more than 2 fold (Table

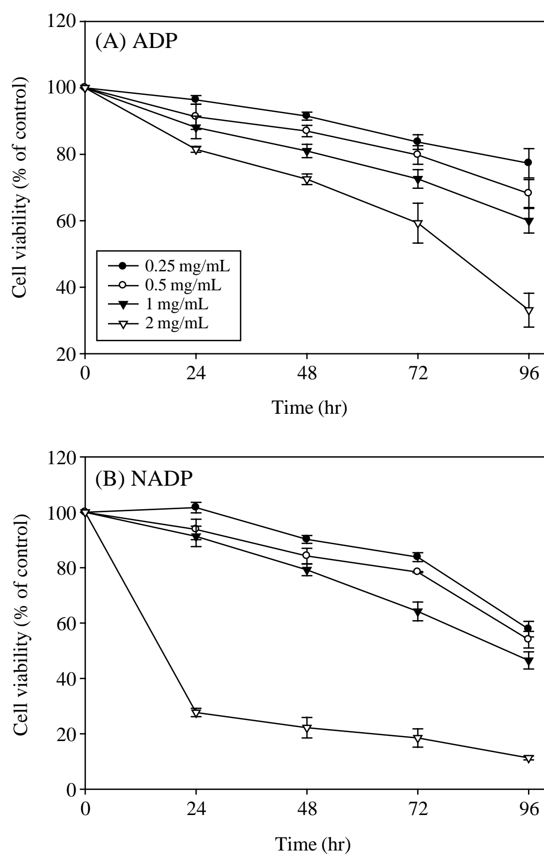

Fig. 4. Cytotoxicity of particle preparations in cultured BEAS-2B cells. Cell viability was assessed by MTT assays, and the results are presented as the percentage of control group viability. Cells were treated with the indicated concentrations of particle preparations for 24, 48, 72, and 96 hours for dose- and time-dependent tests. The results represent the means of three separate experiments. A: ADP (Asian Dust Particles), B: NADP (Non-Asian Dust Particles).

2). The expression of oxidative stress related genes including metallothionein, heme oxygenase-1, heat shock protein 70, and NAD(P)H dehydrogenase 1, were significantly up-regulated.

DISCUSSION

Ambient air particles are generated through both natural and anthropogenic sources. Among these sources, automobile exhaust gases and the use of fossil fuels are important contributors in metropolitan cities (Marconi *et al.*, 2007; O'Connell *et al.*, 2008). Particles occurring in ambient air vary widely in terms of size, composition, morphology, surface charges and other physico-chemical factors. These characteristics may also be affected by meteorological parameters such as temperature, humidity, wind, rainfall, sunlight and others (Rajsić *et al.*, 2004; Rodríquez, *et al.*, 2004; Lee *et al.*, 2006). In recent years, many countries have imposed environmental standards for acceptable levels of particulate matter of the PM_{10} and $PM_{2.5}$ classes. These policies are based on the theory that smaller particles may be more harmful to human health. Many epidemiologists have suggested that the degree of particulate material contamination of the ambient air is closely related to the generation of diseases such as asthma, COPD (Chronic obstructive pulmonary disease), stroke, myocardial infarction, and lung cancer (Koren, 1995; Brändli, 1996; Schwela, 2000). Moreover, the inflammatory diseases caused by particulate matter seem to have seasonal variations. In a previous study, we suggested that variations in the $PM_{2.5}$ level are substantial over the year, while the levels of TSP and PM_{10} show pronounced seasonal variations.

In the present study, we endeavored to identify the distribution of ambient air particles (size: less than 12.1 μm) including nanoparticles in the ambient air in Seoul and also tried to monitor the metal concentrations of the particulates. We also investigated the cytotoxicity and effects on gene expression of the collected particle preparations.

Among the 12 fractions, Fraction 0.52 (size; 0.33～0.52 μm) was the most prevalent during normal days in Seoul but Fraction 2.5 (size; 1.25～2.5 μm) became the main fraction during the Asian dust event. Among the particle fractions collected from 27th March to 3rd April, Fraction 2.5 accounted for 70.2% of the total particles. Kim and Kim reported that TSP/PM_{10} ratios were almost constant, while the $PM_{10}/PM_{2.5}$ or $TSP/PM_{2.5}$ ratios changed noticeably between Asian dust periods and normal dust periods (Kim and Kim, 2003). The present study also showed that $PM_{2.5}$

Table 1. Partial list of genes up-regulated by the treatment of particle preparations

Symbol	Definition	Fold	SD	p value
MT1M	metallothionein 1M	67.49	0.14	0.32
MT1F	metallothionein 1F (functional)	18.66	0.04	0.32
MT1G	metallothionein 1G	16.07	0.08	0.32
MT1X	metallothionein 1X	13.08	0.09	0.32
HMOX1	heme oxygenase (decycling) 1	11.65	0.09	0.45
C20orf127	chromosome 20 open reading frame 127	10.09	0.07	0.36
MMP1	matrix metallopeptidase 1 (interstitial collagenase)	6.20	0.10	0.47
OKL38	pregnancy-induced growth inhibitor (OKL38)	6.01	0.13	0.46
IL24	interleukin 24, transcript variant 2	4.86	0.15	0.59
SQSTM1	sequestosome 1	4.00	0.20	0.44
SGNE1	secretory granule, neuroendocrine protein 1 (7B2 protein)	3.76	0.22	0.54
HSPA1A	heat shock 70 kDa protein 1A	3.70	0.98	0.56
SLC30A2	solute carrier family 30 (zinc transporter), member 2	3.49	0.07	0.40
HSPA1B	heat shock 70 kDa protein 1B	3.03	0.77	0.56
FTH1	ferritin, heavy polypeptide 1	2.93	0.08	0.40
KCNG1	potassium voltage-gated channel, subfamily G, member 1	2.92	0.03	0.42
EGR1	early growth response 1	2.90	0.06	0.45
DNAJA4	DnaJ (Hsp40) homolog, subfamily A, member 4	2.83	0.52	0.52
GCLM	glutamate-cysteine ligase, modifier subunit	2.78	0.02	0.49
NQO1	NAD(P)H dehydrogenase, quinone 1	2.74	0.15	0.46
MT1E	metallothionein 1E (functional)	2.66	0.08	0.45
LOC88523	CG016 (LOC88523)	2.64	0.20	0.47
KIAA1913	KIAA1913	2.52	0.25	0.46
ESM1	endothelial cell-specific molecule 1	2.48	0.25	0.60
ZFAND2A	zinc finger, AN1-type domain 2A	2.44	0.55	0.55
TFPI2	tissue factor pathway inhibitor 2	2.39	0.24	0.54
INSIG1	insulin induced gene 1	2.31	0.08	0.44
TXNRD1	thioredoxin reductase 1	2.29	0.02	0.47
PANX2	pannexin 2	2.28	0.30	0.48
C16orf28	chromosome 16 open reading frame 28	2.26	0.10	0.47
PLA2G4C	phospholipase A2, group IVC (cytosolic, calcium-independent)	2.23	0.09	0.51
PIR	pirin (iron-binding nuclear protein)	2.21	0.09	0.48
HKDC1	hexokinase domain containing 1	2.19	0.30	0.48
GLS	glutaminase	2.18	0.06	0.52
CYP2R1	cytochrome P450, family 2, subfamily R, polypeptide 1	2.18	0.06	0.67
FOLR3	folate receptor 3 (gamma)	2.14	0.16	0.46
TNFRSF6B	tumor necrosis factor receptor superfamily, member 6b	2.14	0.13	0.73
PPIF	peptidylprolyl isomerase F (cyclophilin F)	2.14	0.13	0.47
TRIB3	tribbles homolog 3 (Drosophila)	2.13	0.08	0.40
ETV4	ets variant gene 4 (E1A enhancer binding protein, E1AF)	2.10	0.17	0.46
CTSL	cathepsin L	2.06	0.11	0.44
DDIT3	DNA-damage-inducible transcript 3	2.04	0.03	0.52
CXCL5	chemokine (C-X-C motif) ligand 5	2.02	0.12	0.56
MBP	myelin basic protein	2.00	0.15	0.45
GYPC	glycophorin C (Gerbich blood group)	2.00	0.05	0.62

increased during an Asian dust event.

The total concentration of ambient particles was lower in summer compared to that in spring. This was due to the continuous summer rainfall as well as

Table 2. Partial list of genes up-regulated by the treatment of particle preparations

Symbol	Definition	Fold	SD	p value
NPPB	natriuretic peptide precursor B	−10.16	1.18	0.55
CMKOR1	chemokine orphan receptor 1	−5.56	0.19	0.55
OLR1	oxidised low density lipoprotein (lectin-like) receptor	−4.92	0.69	0.55
DIO2	deiodinase, iodothyronine, type II, transcript variant 3	−4.04	0.32	0.53
EFNA1	ephrin-A1, transcript variant 1	−3.97	0.54	0.51
GAS1	growth arrest-specific	−3.93	0.21	0.46
CCL20	chemokine (C-C motif) ligand 20	−3.77	0.06	0.54
ID1	inhibitor of DNA binding 1, dominant negative helix-loop-helix protein (ID1), transcript variant 2, mRNA	−3.52	0.09	0.46
LOC144501	hypothetical protein LOC144501	−3.43	0.14	0.46
BHLHB2	basic helix-loop-helix domain containing, class B, 2	−3.10	0.21	0.60
CRISPLD2	cysteine-rich secretory protein LCCL domain containing 2	−3.05	0.31	0.48
OXTR	oxytocin receptor	−3.01	0.50	0.63
ATOH8	atonal homolog 8 (Drosophila)	−2.89	0.12	0.45
PPP2R2B	protein phosphatase 2 (formerly 2A), regulatory subunit B	−2.88	0.41	0.60
GJA1	gap junction protein, alpha 1, 43 kDa (connexin 43)	−2.83	0.21	0.56
CD24	CD24 antigen (small cell lung carcinoma cluster 4 antigen)	−2.83	0.25	0.45
LFNG	lunatic fringe homolog (Drosophila)	−2.83	0.08	0.55
OSAP	ovary-specific acidic protein	−2.81	0.10	0.46
DHRS3	dehydrogenase/reductase (SDR family) member 3	−2.80	0.26	0.54
KLF10	Kruppel-like factor 10, transcript variant 1	−2.77	0.07	0.64
CXCL6	chemokine (C-X-C motif) ligand 6	−2.75	0.27	0.47
EDN1	endothelin 1 (EDN1), mRNA.	−2.68	0.44	0.69
CXCL1	chemokine (C-X-C motif) ligand 1	−2.58	0.21	0.45
RGMB	RGM domain family, member B, transcript variant 2	−2.55	0.21	0.66
KLF10	Kruppel-like factor 10, transcript variant 1	−2.51	0.06	0.60
IL6	interleukin 6 (interferon, beta 2)	−2.51	0.50	0.62
FST	follistatin, transcript variant FST344	−2.47	0.08	0.71
HCP5	HLA complex P5	−2.45	0.57	0.62
ID1	inhibitor of DNA binding 1, dominant negative helix-loop-helix protein, transcript variant 2	−2.45	0.07	0.42
SH3RF2	SH3 domain containing ring finger 2	−2.43	0.51	0.59
SLC39A10	solute carrier family 39 (zinc transporter), member 10	−2.38	0.21	0.45
SDPR	serum deprivation response (phosphatidylserine binding protein)	−2.37	0.18	0.45
CCL2	chemokine (C-C motif) ligand 2	−2.30	0.19	0.46
PER2	period homolog 2 (Drosophila), transcript variant 1	−2.28	0.19	0.48
C5orf13	chromosome 5 open reading frame 13 (C5orf13), mRNA.	−2.27	0.26	0.46
KIAA0367	KIAA0367	−2.24	0.70	0.60
PPP1R3C	protein phosphatase 1, regulatory (inhibitor) subunit 3C	−2.24	0.33	0.50
BDKRB1	bradykinin receptor B1	−2.23	0.24	0.48
TNFAIP3	tumor necrosis factor, alpha-induced protein 3	−2.22	0.08	0.55
HAS3	hyaluronan synthase 3, transcript variant 1	−2.22	0.26	0.70
SMAD6	SMAD, mothers against DPP homolog 6 (Drosophila)	−2.21	0.04	0.46
WDR69	WD repeat domain 69	−2.16	0.11	0.70
PRSS7	protease, serine, 7 (enterokinase)	−2.16	0.17	0.66
CNN2	calponin 2, transcript variant 1	−2.15	0.41	0.56
CPA4	carboxypeptidase A4	−2.13	0.91	0.65
CSF2	colony stimulating factor 2 (granulocyte-macrophage)	−2.08	0.40	0.57
GPRC5A	G protein-coupled receptor, family C, group 5, member A	−2.07	0.31	0.51
AOX1	aldehyde oxidase 1	−2.05	0.52	0.61

Table 2. Continued.

Symbol	Definition	Fold	SD	p value
DOC1	downregulated in ovarian cancer 1, transcript variant 2	−2.05	0.76	0.62
CYFIP2	cytoplasmic FMR1 interacting protein 2	−2.05	0.01	0.67
C6orf32	chromosome 6 open reading frame 32	−2.02	0.06	0.44
MAP3K8	mitogen-activated protein kinase	−2.01	0.12	0.45
THBS1	thrombospondin 1	−2.00	0.23	0.58
SLITL2	slit-like 2 (Drosophila)	−2.00	0.25	0.56
CAST	calpastatin, transcript variant 1	−2.00	0.37	0.56

the decrease in use of fossil fuel for heating. However, the particle size in summer tended to increase. The main fraction changed to 0.52 ~ 0.76 μm in summer from 0.33 ~ 0.52 μm in spring. This phenomenon may be attributable to the climate in summer, which is humid, warm, and windless; this provides high humidity and temperature conditions that are conducive to rapid growth of particles (Zimmer, 2002; Rajsić *et al.*, 2004; de Hartog *et al.*, 2005; Karar *et al.*, 2006; Lee *et al.*, 2006) (Fig. 1).

The content of Mn and Fe in air particles is known to have a soil origin and these elements were significantly increased during the Asian dust event. However, the concentrations of As and Pb, which are known to be anthropogenic, did not fluctuate even during the Asian dust event. Lee *et al.* also suggested that concentration of Mn, Fe, Ni, and Cr were significantly increased during Asian dust storms, while the concentration of Pb, Cd, and Co was not affected by these disturbances (Lee *et al.*, 2006).

The cytotoxicity of the NADP particle was more than 3.5 fold that of the ADP particles. In the ADP preparation, cytotoxic hazardous chemicals seemed to be lower than they were in the NADP preparation, probably because Asian dust originated from non-polluted areas of the Chinese mainland. Although some toxic chemicals may be attached to the Asian dust when it flies to the eastern parts of Asia, the primary distribution seemed to be a non-contaminated soil fraction. In contrast, the NADP preparation may have contained many anthropogenic hazardous materials attached to the particles and this could explain its higher cytotoxicity (Fig. 4).

Ambient particles trigger the defense mechanisms of immune systems against oxidative stress. Microarray analysis confirmed this phenomenon. Metallothioneins are cysteine-rich metal-binding proteins with known antioxidant properties. In the present study, metallothioneins 1M, 1F, 1G, and 1X were up-regulated 67.49, 18.66, 16.07, and 13.08 fold, respectively, by NADP extracts (Table 1). Expression of metallothionein-1 was increased 2.16 fold in a rat testis injected with 4 μmol/kg cadmium (Mukhopadhyay *et al.*, 2009).

Heme oxygenase-1 serves a vital metabolic function as the rate-limiting step in the heme degradation step in the heme catabolic pathway and is important in the maintenance of iron homeostasis. Bilirubin, a potent antioxidant, arises from enzymatic reduction of biliverdin, a product of heme oxygenase activity, by biliverdin reductase. Expression of heme oxygenase-1 can be up-regulated by various stresses including oxidative stress, heat shock, endotoxins, and ultraviolet irradiation (Baranano *et al.*, 2002; Yasui and Akagi, 2007; Schafer and Werner, 2008; Morse *et al.*, 2009). Heme oxygenase-1 was increased about 15.95 fold in arsenic-treated rat cardiomyocytes (Park and Park, 2006) and is known as a potent biomarker for hexavalent chromium-induced stress and toxicity in human dermal fibroblasts (Joseph *et al.*, 2008). Up-regulation of metallothionein-2A and heme oxygenase-1 also was observed following exposure to gallium nitrate (Yang and Chitambar, 2008). The changes in gene expression shown in Table 1 and Table 2 in the present study suggest that inhalation of ambient particles could cause serious inflam-

matory diseases triggered by oxidative stress and tissue damage.

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