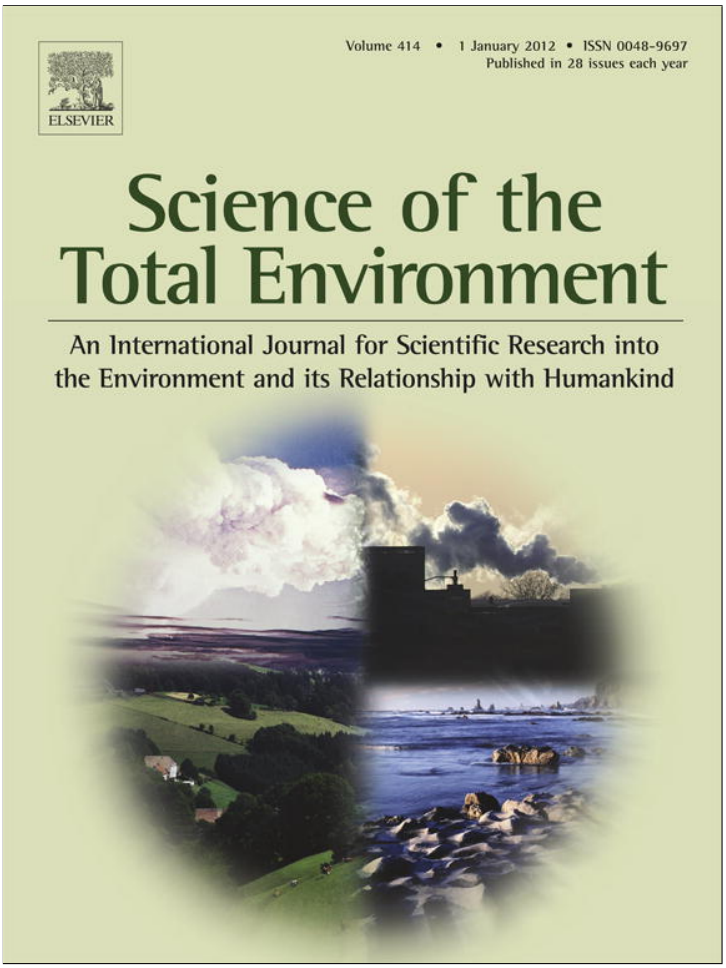

Science of the Total Environment 414 (2012) 68–72

Contents lists available at SciVerse ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Effects of temple particles on inflammation and endothelial cell response

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ARTICLE INFO

Article history:
Received 12 February 2011
Received in revised form 13 August 2011
Accepted 17 August 2011
Available online 21 November 2011

Keywords:
Temple particles
Polycyclic aromatic hydrocarbons
Human coronary artery endothelial cell

ABSTRACT

To pray in temples is a regular activity in Buddhism and Taoism societies, yet few studies investigated the effects of particles from incense-burning in temples. The objectives of this study are to examine particle size and polycyclic aromatic hydrocarbon (PAH) effects of particles on coronary artery endothelial cell. We used two micro-orifice uniform deposit impactors to collect 11 sets of particles at a Chinese temple in Yi-Lan, Taiwan. 16 PAHs were determined by a high-resolution gas chomatograph/high-resolution mass spectrometer. Human coronary artery endothelial cells were exposed to particle extracts in three size ranges: $PM_{0.1}$ (diameters less than 0.1 μm), $PM_{1.0–0.1}$ (diameters between 1.0 and 0.1 μm), and $PM_{10–1.0}$ (diameters between 10 and 1.0 μm) at 50 μg/mL for 4 h, and interleukin-6 (IL-6), endothelin-1 (ET-1), and nitric oxide (NO) concentrations in the medium were measured. We found that $PM_{1.0–0.1}$ stimulation resulted in significantly higher IL-6 and ET-1 production than $PM_{0.1}$ and $PM_{10–1.0}$. $PM_{1.0–0.1}$ also significantly reduced HCAEA cells to synthesize NO. Naphthalene, acenaphthylene, acenaphthene and anthracene of $PM_{1.0–0.1}$ were highly correlated with NO reduction. This study found that size and composition of temple particles were both important factors in inducing cytokine production and reducing NO formation in human coronary artery endothelial cell cultures.

1. Introduction

It is already known that exposure to ambient particulate matter (PM) is associated with adverse health effects (Dockery and Pope, 1996), which is considered as a risk factor for cardiovascular morbidity (Dominici et al., 2006) and mortality (Pope et al., 2004a). Previous study showed that a temple has a higher level of PM concentration than outdoors, which is a result of incense-burning (Lung et al., 2004). Burning incense is a long-lasting Chinese tradition to give respect to ancestors for deity worship in Buddhism and Taoism. A previous study showed that 40% of 121 Taiwan households worship twice a day and are exposed to high levels of particulate air pollution (Lung et al., 1999).

The prominent hypotheses linking particulate matter (PM) with cardiovascular diseases involve direct effects of PM on lung receptor, heart and blood, and/or indirect effects mediated through pulmonary oxidant stress and inflammation secondary to blood coagulation, endothelial dysfunction and autonomic dysfunction (Brook et al., 2010). A recent study suspected that PM-modulated endothelial dysfunction may play a central role in the pathogenesis of cardiovascular diseases among these pathophysiologic pathways (Rajagopalan et al., 2005). The endothelium is crucially involved in the regulation of coronary blood flow and cardiac functions, such as coronary heart disease, heart failure, diabetes and hypertension (Brunner et al., 2005).

Previous study reported that exposure to ambient PM was negatively associated with endothelial dysfunction in patients with diabetes (O'Neill et al., 2005). An in vivo study showed that polycyclic aromatic hydrocarbons (PAHs), the common environmental contaminants usually formed by pyrolysis of organic matter during combustion of fossil fuels in urban air (Arfstenm et al., 1996) and incense burning in temples (Lung and Kao, 2003), could induce the vasorelaxation effect by activation of nitric oxide synthesis of endothelium (Kang and Cheng, 1997). However, no study has shown in vitro the association between the size and PAHs of PM and endothelial function by coronary artery endothelial cell.

To examine the size and PAH effects of particles on coronary artery endothelial cell, particle samples at a major Chinese temple in three size ranges, $PM_{0.1}$ (diameters less than 0.1 μm), $PM_{1.0–0.1}$ (diameters between 1.0 and 0.1 μm), and $PM_{10–1.0}$ (diameters between 10 and 1.0 μm), were extensively characterized and correlated with cytokine-inducing and endothelial dysfunction in coronary artery endothelial cell. The purpose of this study is to investigate size distribution and concentrations of particulate and particle-phase polycyclic aromatic

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0048-9697/$ – see front matter © 2011 Published by Elsevier B.V.
doi:10.1016/j.scitotenv.2011.08.050

hydrocarbons (PAHs) measured in the temple (temple particles) and to determine the relationship between inflammatory reaction and endothelial dysfunction with temple particles.

2. Materials and methods

2.1. Temple particle collection

We used two Micro-Orifice Uniform Deposit Impactors (MOUDI™; MSP Inc., Minneapolis, MN, USA) with a flow rate of 30 L/min to collect temple particles with a size range between 0.05–0.1, 0.10–0.18, 0.18–0.32, 0.32–0.56, 0.56–1.0, 1.0–1.8, 1.8–3.2, 3.2–5.6, 5.6–10, 10–18 and >18 μm. Temple particles were collected on 11 37-mm Alumni filters in one sampler for PAH extraction and analysis, and also collected on 11 37-mm Teflon filters in the other one sampler for in vitro assays of temple particle bioactivity. We collected temple particles at a Taoist temple, which is located in Yi-Lan county, putting the samplers 5 m away from a major incense burner in the temple. A total of 26 sets of temple particle samples (286 filters) were collected by two samplers (13 sets by each sampler) between 0630 h and 2130 h (temple's opening hours) during 5–9 July 2010 (five days) and 2–9 August 2010 (eight days).

2.2. PAH extraction and analysis

All Teflon filters were extracted by three 15-min sonications with 10 ml of hexane: methylene chloride mixture (volume ratio 1:4). Extracts were concentrated down to about 1 ml under a gentle stream of nitrogen, cleaned up by methylene-chloride-soaked silicon gel (70–230 mesh, for column chromatography, Macherey-Nagel GmbH & Co., Duren, Germany), and eluted by 25 ml hexane and 25 ml hexane: methylene chloride (volume ratio 3:2) mixtures. Elutes were concentrated again to about 200 ml to be analyzed. Samples were analyzed for 16 PAHs, which are listed as priority pollutants by USEPA, (1982). They are naphthalene (Nap), acenaphthylene (Acy), acenaphthene (Acp), fluorine (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Fl), pyrene (Pyr), benz[*a*]anthracene (B[*a*]A), chrysene (Chry), benzo[*b*]fluoranthene (B[*b*]F), benzo[*k*]fluoranthene (B[*k*]F), benzo[*a*]pyrene (B[*a*]P), indeno[1,2,3-*c*,*d*]pyrene (Ind), dibenz[*a*,*h*]anthracene (DBA), and benzo[*g*,*h*,*i*]perylene (B[ghi]P). Analytical standards of 16 PAH mixtures (PM-611), 6 deuterated-PAH mixtures (US-108N) and Benz[*a*]anthracene-d_{12} (IST-120, used as internal standard) were obtained from Ultra Scientific, North Kingston, Rhode Island. The purity of all standards were 99% or higher. All solvents were of UltraResi-Analyzed quality. They were purchased from Millinckrodt Baker Inc. Paris, Kentucky. PAH analysis was performed with a high-resolution gas chomatograph/high-resolution mass spectrometer (HRGC/HRMS). The HRGC (Hewlett Packard 6970 Series gas, CA, USA) was equipped with a DB-5MS fused silica capillary column (L = 60 m, ID = 0.25 mm, film thickness = 0.25 μm) (J&Wi Scientific, CA, USA), and with a splitless injection. Helium was used as the carrier gas. The HRMS (Micromass Autospec Ultima, Manchester, UK) was equipped with a positive electron impact (EI+) source. The analyzer mode of the selected ion monitoring (SIM) was used with resolving power of 10,000. The electron energy and source temperature were specified at 35 eV and 250 °C, respectively. Individual PAH was quantified via the internal standard method.

2.3. Quality assurance and quality control

Thirteen laboratory blanks and 13 field blanks were used to assess contamination that occurred in the laboratory and during assembling and transportation processes. They were processed as real samples and taken to the field except for the connected pumps not being turned on. Blank values of 16 individual PAHs were less than 10 ng. The percentage of recovery rate of the 16 PAHs were about 80.4 (Acy)-97.8 (B[*b*]F)%.

2.4. Particle suspension preparation

All 37-mm Alumni filters were equilibrated in 50 ± 5% relative humidity for more than 48 h and weighed before and after air sampling to obtain temple particle mass. The filters were submerged in 2.5 mL endotoxin-free water (Sigma, St. Louis, MO, USA) and sonicated in water bath (Bandelin Sonorex, Möerfelden-Walldorf, Germany) for 30 min to extract particles. After sonication, the filters were weighed again to determine the weight of water-extractable particles in the samples. Accordingly, the particle suspension contained particles either dissolved or suspended in water. The suspensions of particles with size ranges between 1.0–1.8, 1.8–3.2, 3.2–5.6 and 5.6–10 μm were combined to represent $PM_{1.0–10}$ while those with size ranges between 0.10–0.18, 0.18–0.32, 0.32–0.56 and 0.56–1.0 μm were combined to represent $PM_{1.0–0.1}$. The suspension of particles with a size range between 0.05 and 0.10 was used as $PM_{0.1}$. The particle extraction fractions were 52 ± 14% for $PM_{0.1}$, 45 ± 13% for $PM_{1.0–0.1}$, and 80 ± 12% for $PM_{10–1.0}$. The particle suspension was stored at −20 °C and sonicated for 1 min before cell stimulation. In total, there were 143 samples for in vitro assay.

2.5. In vitro assays of particle bioactivity

Human coronary artery endothelial cells (HCAEC) (300-05A; Cell Applications, Inc., San Diego, CA, USA) were maintained in endothelial cell basal medium (210–500; Cell Applications, Inc.) and supplemented with endothelial cell growth medium (212–500; Cell Applications, Inc.) into a T-75 flask. For exposure experiments, HCAEC cells at 1.0×10^5 cells/mL were seeded onto 6-well tissue culture plates (Techno Plastic Products AG, Trasadingen, Switzerland) in triplicates and cultured for 48 h. The medium was then changed to endothelial cell basal medium (210–500; Cell Applications, Inc.) containing 0, 25 or 50 μg/mL of temple particles. The supernatant was collected 4 h later and kept at −80 °C for measuring interleukin-6 (IL-6), endothelin-1 (ET-1) and nitric oxide (NO) concentrations. Viability of cells was examined by trypan blue exclusion.

2.6. Determination of interleukin-6

IL-6 was determined using an enzyme linked immunosorbent assay (ELISA) kit (R&D systems, Inc., Minneapolis, MN, USA). A monoclonal antibody specific for rat IL-6 was coated on a 96-well polystyrene microplate. A polyclonal antibody against rat IL-6 conjugated to horseradish peroxidase (HRP) was used as the IL-6 conjugate. Recombinant IL-6 ranging from 62.5 to 2000 pg/mL was used as the standard. The minimum detectable concentration of IL-6 in this assay ranged from 14 to 36 pg/mL. All procedures followed the manufacturer's recommendation.

2.7. Determination of endothelin-1

ET-1 was determined using an ELISA kit (R&D Systems, Inc.). A murine monoclonal antibody against ET-1 was precoated on a microplate, and monoclonal antibody ET-1 conjugated to HRP was used as the ET-1 conjugate. Synthetic human ET-1 ranging from 0.32 to 1000 pg/mL was used as standards. The minimum detectable dose of ET-1 was less than 0.16 pg/mL. All procedures followed the manufacturer's recommendation.

2.8. Determination of nitric oxide

The NO production was determined using a Nitric Oxide Synthase Assay Kit, Colorimetric (Calbiochem Inc.). The final products of NO in vivo are nitrite [NO_2] and nitrate [NO_3]. This assay uses the sum of [NO_2] and [NO_3] as the index of total NO production. A total of 40 mL of ultrafiltered plasma sample reacted with 1 mM NADPH and nitrate reductase for 60 min at room temperature. Then 10 ml

of cofactors and LDH was added and incubated for 20 min. Subsequently, Griess reagents R1 and R2 were added to develop color for 10 min, and the absorbance was read at 540 nm. Determination of [nitrate + nitrite] concentration was adjusted with the standard curve of nitrate according to the manufacturer's equation.

2.9. Statistical analysis

To compare IL-6, ET-1 and NO induced by blank, $PM_{0.1}$, $PM_{1.0–0.1}$, and $PM_{10–1.0}$ samples, the one-way analysis of variance (ANOVA) with the Scheffe mean comparison test was used. The quantile–quantile plot was used to test for normal distribution of the data. Pearson's correlation coefficient (R) was used to evaluate the relations between IL-6, ET-1, NO, and PAHs. The level of significance for all statistical analyses was chosen as $p<0.05$. All statistical analyses were made using SPSS software (version 11.0; SPSS Inc., Chicago, IL, USA).

3. Results

As shown in Fig. 1, the majority of temple particles (Fig. 1a) and total PAHs (Fig. 1b) in temple particles was composed of submicrometer particles within 0.1 to 1.0 μm. The mean concentrations (standard deviation, SD) of $PM_{10–1.0}$, $PM_{1.0–0.1}$ and $PM_{0.1}$ were 31.0 (6.3), 119.0 (35.2) and 4.8 (3.1) μg/m^3. The total PAHs in $PM_{10–1.0}$, $PM_{1.0–0.1}$ and $PM_{0.1}$ were 0.07 (0.002), 0.19 (0.002) and 0.02 (0.001) μg/m^3, respectively. Table 1 shows the mass concentrations of 16 PAHs in $PM_{0.1}$, $PM_{1.0–0.1}$ and $PM_{10–1.0}$. 16 PAH concentrations were highest in $PM_{1.0–0.1}$ and the contents of these PAHs were significantly different among the three particle size ranges.

We found that PM with different size ranges at 25 or 50 μg/mL stimulated HCAEC cells to produce IL-6 and ET-1, and to reduce NO formation. The concentrations of IL-6, ET-1 and NO were not significantly different between 25 and 50 μg/mL. The viability of HCAEC cells after the incubation with particle extracts at 50 μg/mL for 4 h was 85–94%, which was not significantly different from that of untreated cells.

Particles of the three size ranges could significantly stimulate HCAEA cells to produce IL-6 and ET-1 (ANOVA, $p<0.05$) (Fig. 2). $PM_{1.0–0.1}$ significantly stimulated the highest production of IL-6 and ET-1, which was 2-fold higher than that of blank filters. $PM_{10–1.0}$ and $PM_{0.1}$ could also elicit less than double the IL-6 production of blank filters. The significant difference of IL-6 and ET-1 production was found between $PM_{10–1.0}$, $PM_{1.0–0.1}$ and $PM_{0.1}$. For the comparison of IL-6 and ET-1 production induced by particles of three size ranges at 50 μg/mL, $PM_{1.0–0.1}$ could significantly stimulate HCAEA cells to produce higher IL-6 and ET-1 (ANOVA, $p<0.05$) than that for $PM_{10–1.0}$ and $PM_{0.1}$. For NO concentration, exposure of HCAEA cells to $PM_{1.0–0.1}$ markedly reduced NO formation (ANOVA, $p<0.05$). On the contrary, $PM_{10–1.0}$ and $PM_{0.1}$ could activate HCAEA cells to synthesize higher NO than that for blank.

Because $PM_{1.0–0.1}$ induced the most significant IL-6 and ET-1 production and decreased NO level, we limit the following analysis to this size fraction. Correlation analysis was used to examine whether any of the PAH components were associated with the biological end points (Table 2). Among the 16 PAHs, Nap, Acy, Acp, Flu, and Ant were highly correlated with NO with statistical significance. No significant correlation was observed between PAHs and the other biological end points.

4. Discussion

This is the first study on the size and PAH effects of temple particles on HCAEC. Particles collected by a MOUDI sampler in a major Taoist temple were extensively characterized to identify PAHs responsible for bioactivity. In this study, we found that exposure of HCAEC to temple particles/PAHs causes significant increases in IL-6 and ET-1, and decreases in NO. These findings provide partial support for previous epidemiological studies showing the association between PM exposure, inflammation (Pope et al., 2004b) and endothelial dysfunction (O'Neill et al., 2005) among humans.

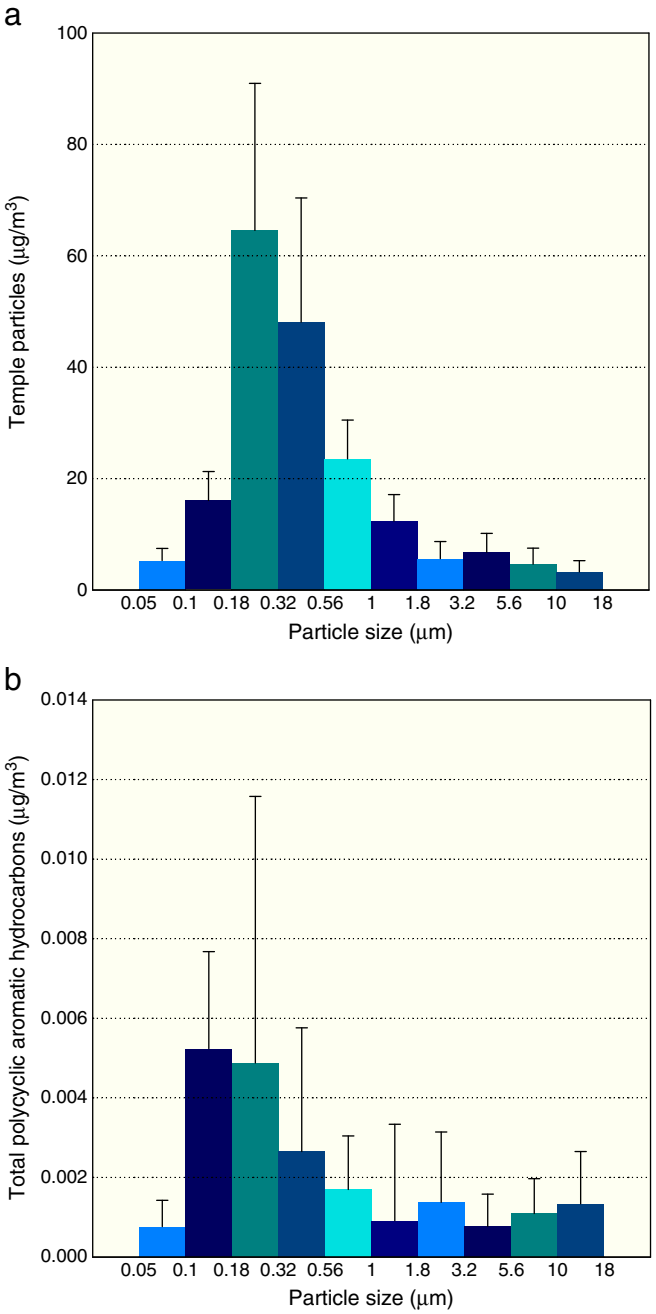

Fig. 1. Particle size distributions of (a) temple particles and (b) total polycyclic aromatic hydrocarbons in temple particles.

Consistent with our results, a recent in vivo study reported that traffic-related $PM_{2.5}$ exposure was associated with IL-6 and ET-1 production and NO reduction (Lei et al., 2005) in diabetic rats. Another in vivo study showed that concentrated ambient particle exposure was associated with elevated ET-1 in small pulmonary arteries in rats with chronic bronchitis (Batalha et al., 2002). Two in vitro studies also showed that exposure of endothelial cells to diesel exhaust particles reduced NO formation (Bai et al., 2001; Ikeda et al., 1998). Contrarily, one in vitro study demonstrated that exposure of endothelial cells to PAHs was associated with increased NO production (Kang and Cheng, 1997).

Table 1
Summary statistics for the temple particles and polycyclic aromatic hydrocarbons (PAHs) (n = 13) of three particle sizes (mean ± SD).

	$PM_{10-1.0}$	$PM_{1.0-0.1}$	$PM_{0.1}$	ANOVA	
				P-value	Scheffe test
Temple particles, μg/m^3	31.0 ± 6.3	119.0 ± 35.2	4.8 ± 3.1	0.01	$PM_{10-1.0}/PM_{1.0-0.1}/PM_{0.1}$
Total PAHs in temple particles, μg/m^3	0.07 ± 0.002	0.19 ± 0.002	0.02 ± 0.001	0.01	$PM_{10-1.0}/PM_{1.0-0.1}/PM_{0.1}$
16 PAHs in temple particles, ng/m^3					
Naphthalene	17.0 ± 1.6	23.0 ± 2.1	2.4 ± 1.1	0.01	$PM_{10-1.0}/PM_{1.0-0.1}/PM_{0.1}$
Acenaphthylene	2.2 ± 0.2	4.0 ± 0.9	0.5 ± 0.2	0.01	$PM_{10-1.0}/PM_{1.0-0.1}/PM_{0.1}$
Acenaphthene	1.7 ± 0.2	2.5 ± 0.4	0.4 ± 0.3	0.02	$PM_{10-1.0}/PM_{1.0-0.1}/PM_{0.1}$
Fluorine	1.4 ± 0.9	2.9 ± 0.6	0.2 ± 0.1	0.01	$PM_{10-1.0}/PM_{0.1}$; $PM_{1.0-0.1}/PM_{0.1}$
Phenanthrene	2.0 ± 1.1	4.7 ± 0.9	0.3 ± 0.1	0.01	$PM_{10-1.0}/PM_{1.0-0.1}/PM_{0.1}$
Anthracene	0.5 ± 0.1	0.8 ± 0.4	0.2 ± 0.1	0.02	$PM_{10-1.0}/PM_{0.1}$; $PM_{1.0-0.1}/PM_{0.1}$
Fluoranthene	0.9 ± 0.3	8.4 ± 1.4	0.2 ± 0.1	0.01	$PM_{10-1.0}/PM_{1.0-0.1}/PM_{0.1}$
Pyrene	1.0 ± 0.3	10.1 ± 2.6	0.3 ± 0.1	0.01	$PM_{10-1.0}/PM_{1.0-0.1}/PM_{0.1}$
Benz[*a*]anthracene	0.5 ± 0.3	17.5 ± 9.8	0.3 ± 0.2	0.01	$PM_{10-1.0}/PM_{1.0-0.1}$
Chrysene	0.8 ± 0.4	22.0 ± 12.3	0.5 ± 0.3	0.01	$PM_{10-1.0}/PM_{1.0-0.1}$; $PM_{1.0-0.1}/PM_{0.1}$
Benzo[*b*]fluoranthen	3.8 ± 1.3	46.0 ± 26.4	1.9 ± 0.5	0.01	$PM_{10-1.0}/PM_{1.0-0.1}/PM_{0.1}$
Benzo[*k*]fluoranthene	2.9 ± 2.2	12.0 ± 5.1	0.5 ± 0.2	0.01	$PM_{10-1.0}/PM_{1.0-0.1}/PM_{0.1}$
Benzo[*a*]pyrene	4.8 ± 4.3	26.2 ± 12.1	0.8 ± 0.3	0.01	$PM_{10-1.0}/PM_{1.0-0.1}/PM_{0.1}$
Indeno[1,2,3-*c*,*d*]pyrene	2.9 ± 2.1	23.5 ± 11.3	0.7 ± 0.6	0.01	$PM_{10-1.0}/PM_{1.0-0.1}/PM_{0.1}$
Dibenz[*a*,*h*]anthracene	3.7 ± 2.2	9.3 ± 2.2	0.4 ± 0.1	0.01	$PM_{1.0-10}/PM_{1.0-0.1}/PM_{0.1}$
Benzo[*g*,*h*,*i*]perylene	1.3 ± 0.4	23.8 ± 8.5	0.8 ± 0.2	0.01	$PM_{1.0-10}/PM_{1.0-0.1}$; $PM_{1.0-0.1}/PM_{0.1}$

Increased concentrations of IL-6 are associated with an increased risk of cardiovascular events (Lindmark et al., 2001). The association between elevated PM levels and increased serum IL-6 has also been reported in humans (van Eeden et al., 2001). IL-6 is directly involved in regulation of the synthesis of C-reactive protein in the liver. CRP is a sensitive indicator of infection, injury, and inflammation and is linked to increased risk of cardiovascular disease (Ridker, 2001), and its concentration has been shown to be positively associated with exposure to particulate air pollution (Pope et al., 2004a). Increases in inflammatory measures such as C-reactive protein with PM exposure have also been shown and may represent responses to endothelial dysfunction and activation of oxidant stress/cytokine pathways (Peters et al., 2001). Decreased NO level is likely to activate the sympathetic system (Magari et al., 2001) and an elevation in blood pressure (Zanobetti et al., 2004) and this in the intermediate and long term may influence vascular morbidity and mortality. Thus, it is possible that PM exposure through alterations in endothelial function, activation of the sympathetic system, endothelin-1 axis, and platelet function may predispose to hypertension and atherosclerosis (Rajagopalan et al., 2005).

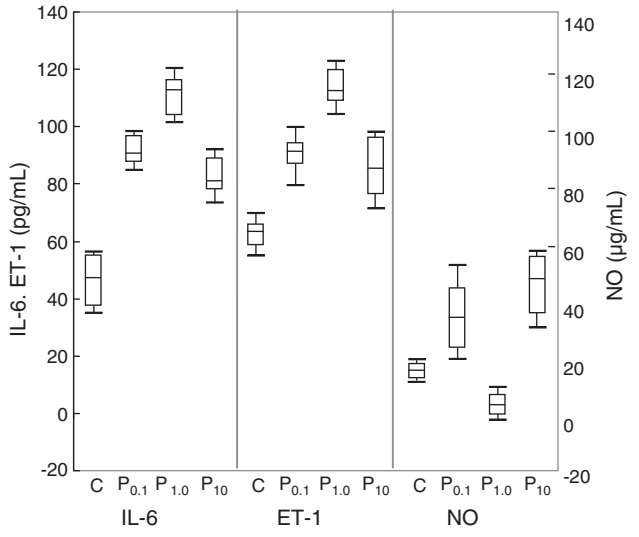

Fig. 2. Comparison of interleukin-6 (IL-6), endothelin-1 (ET-1), and nitric oxide (NO) production induced by 50 μg/mL particles of three size ranges. Each box plot is composed of five horizontal lines displaying the 10th, 25th, 50th, 75th and 90th percentiles. C, $P_{0.1}$, $P_{1.0}$, and P_{10} indicate blank filters, particles with aerodynamic diameter less than 0.1 μm ($PM_{0.1}$), between 1.0 and 0.1 μm ($PM_{1.0-0.1}$), and between 10 and 1.0 μm ($PM_{10-1.0}$), respectively.

Our study confirmed that the ability of particulate pollutant to elicit cytokine production was size dependent, being greatest for submicrometer particles ($PM_{1.0}$). Previous study using different sizes of coal fly ash showed that submicrometer particles resulted in more cytokine production than did large particles (Smith et al., 2000) in lung epithelial A549 cells. A recent study using different sizes of ambient particles reported that $PM_{1.0}$ was more potent in causing cytokine secretion than were fine (diameters between 1 and 2.5 μm; $PM_{1.0-2.5}$), and coarse (diameters between 2.5 and 10 μm; $PM_{2.5-10}$) particles (Huang et al., 2003) in human bronchial epithelial cells. Moreover, the results suggested that NO alteration was associated with certain PAHs, including Nap, Acy, Acp, Flu, and Ant. Such findings are consistent with previous study showing the association of NO release with Nap, Acy, Acp, Flu, and Ant in endothelium of rat aorta (Kang and Cheng, 1997). The underlying mechanism of this effect may be through the Ca^{2+}/calmodulin pathway (Lowenstein and Snyder, 1992). Immunosuppressive anthracene- and pyrene-based PAHs have been shown to increase the concentration of intracellular Ca^{2+} in B cells (Davis and Burchiel, 1992) and T cells (Krieger et al., 1994) possibly

Table 2
Correlation of 16 polycyclic aromatic hydrocarbons in particles with aerodynamic diameter between 1.0 and 0.1 μm ($PM_{1.0-0.1}$) with interleukin-6, nitric oxide and endothelin-1 induced by $PM_{1.0-0.1}$ at 50 μg/mL.

	Interleukin-6	Nitric oxide	Endothelin-1
Naphthalene	−0.60	−0.99*	−0.52
Acenaphthylene	−0.52	−0.90*	−0.54
Acenaphthene	−0.49	−0.90*	−0.42
Fluorine	−0.41	−0.84	−0.58
Phenanthrene	−0.30	−0.55	−0.43
Anthracene	−0.52	−0.97*	−0.70
Fluoranthene	0.54	−0.32	0.12
Pyrene	0.20	−0.60	−0.45
Benz [*a*] anthracene	0.63	−0.59	0.08
Chrysene	0.61	−0.42	0.11
Benzo [*b*] fluoranthene	0.14	−0.70	−0.56
Benzo [*k*] fluoranthene	−0.42	−0.20	−0.12
Benzo [*a*] pyrene	0.18	−0.43	−0.41
Indeno [1,2,3-*c*, *d*] pyrene	0.12	−0.39	−0.36
Dibenz [*a*,*h*] anthracene	−0.60	−0.57	−0.81
Benzo [*g*,*h*,*i*] perylene	0.21	−0.61	−0.35

* p-value < 0.05.

by activation of phospholipase C (PLC) (Archuleta et al., 1993) or inhibition of endoplasmic/sarcoplasmic reticulum Ca^{2+}-ATPase (Krieger et al., 1995).

One major limit of the study is that we didn't measure the elemental contents and endotoxin content of PM, which were reported to be associated with cytokine production (Lindmark et al., 2001; van Eeden et al., 2001). Nonetheless, our results support the hypothesis that PAHs in temple particles play an important role in cytokine induction and reduction.

We presented evidence that exposure of HCAEC to temple particles caused significant alterations in IL-6 and ET-1 and NO. The size and PAHs of temple particles were both important factors in inducing cytokine production.

Acknowledgments

This study was supported by grants (98-2314-B-562-001 and NSC 99-2314-B-562-001-MY2) from the National Science Council of Taiwan.

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