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Enhanced oxidative stress and endothelial dysfunction in streptozotocin-diabetic rats exposed to fine particles

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Abstract

The association between ambient particulate matter (PM) and cardiovascular diseases has been demonstrated in epidemiological studies. Recent studies suggest that diabetic patients are at greater risk for PM-associated cardiovascular events. Although diabetes and PM exposure individually have been reported to be associated with increased oxidative stress, inflammation, and endothelial dysfunction, it is not clear whether PM may induce synergistic interaction effects on these parameters in diabetics. Strepotozotocin-induced diabetic ($n = 4$) and healthy ($n = 4$) rats were intratracheally administered with $PM_{2.5}$ collected from a busy traffic area in a dose of 200 μg suspended in 0.5 mL phosphate-buffered saline (PBS). The same number of rats was exposed to PBS as controls. Cell and differential counts and protein and lactate dehydrogenase activity were determined in bronchoalveolar lavage. Markers of 8-hydroxydeoxy-guanosine (8-OHdG), endothelin-1 (ET-1), and [nitrate + nitrite], an indicator of nitric oxide (NO) production, in addition to C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) in peripheral blood were also determined. Our results showed that diabetic rats were associated with increased 8-OHdG, IL-6, and ET-1 decreased [nitrate + nitrite]. In nondiabetic rats PM exposure was also associated with increased 8-OHdG, IL-6, TNF-α, and CRP but decreased [nitrate + nitrite]. Interestingly, increases of 8-OHdG and ET-1 after PM exposure were more prominent in diabetic rats than in nondiabetic rats. The general linear model further indicated that there were interactions between diabetes and PM on 8-OHdG ($P < 0.01$) and ET-1 ($P = 0.08$). We suggest that PM exposure may enhance the risk of cardiovascular diseases through interaction between PM and diabetes on excess reactive oxygen species generation and endothelial dysfunction. These findings provide further support for previous epidemiological studies.

Keywords: Diabetes; Fine particles; Oxidative stress; Endothelin-1

1. Introduction

The association between ambient particulate matter (PM) and cardiovascular diseases has been demonstrated in epidemiological studies (Pope and Dockery, 1999; Samet et al., 2000; Pope et al., 2002). Subjects with existing cardiovascular diseases including ischemic heart disease and congestive heart failure are found to be more susceptible to ambient PM exposure (Pope et al., 2002). Recently, some researchers have also suggested that diabetic patients are another subpopulation at risk for PM-associated cardiovascular events (Goldberg et al., 2001; Zanobetti and Schwartz, 2001, 2002). Diabetes has been reported to be an effect modifier of air-pollution-related hospital admissions for heart disease in elderly persons (Zanobetti and Schwartz, 2001) and has been demonstrated to double the risk of PM_{10}-associated cardiovascular admissions compared

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to those of nondiabetics (Zanobetti and Schwartz, 2002). Diabetes mellitus is a common disease and has been associated with cardiovascular morbidity and mortality (Resnick and Howard, 2002). PM is also reported to affect cardiovascular diseases (Pope et al., 2004). However, the mechanisms through which PM enhances the risks of cardiovascular disease in diabetics remain unclear. We hypothesize that diabetes and PM may share common pathways and interact in the development of cardiovascular events.

Hyperglycemia of diabetics has been associated with increased reactive oxygen species (ROS) formation (Maritim et al., 2003). Diabetic patients usually have significantly elevated concentrations of 8-hydroxydeoxyguanosine (8-OHdG) in their serum (Nishikawa et al., 2003) and decreased levels of glutathione (GSH) (Dincer et al., 2003). It has been proposed that increased ROS may induce inflammation in endothelium, alter endothelium function, and increase coagulability (Beckman et al., 2002). Inflammation activity also increased in individuals with diabetes, as shown by increased levels of C-reactive protein (CRP) (Jager et al., 1999; Schalkwijk et al., 1999), interleukin-6 (IL-6) (Schram et al., 2003), and tumor necrosis factor (TNF-α) (Lechleitner et al., 2000). Hyperglycemia also inhibits the production of nitric oxide (NO) by blocking endothelial NO synthase (eNOS) activation and increasing the production of ROS in endothelial and vascular smooth muscle cells (De Vriese et al., 2000). In addition to reducing concentration of NO, diabetes increases the production of vasoconstrictors, most importantly, endothelin-1 (ET-1). Reports on endothelial dysfunction in patients with diabetes have been widely studied, including increased ET-1 and decreased NO (Haak et al., 1992; Williams et al., 1996).

The exact mechanisms through which ambient PM causes cardiovascular diseases remain unclear. PM exposure is associated with increased generation of ROS (Tao et al., 2003). It is proposed that oxidative stress in peripheral blood induced by PM may be potentially related to cardiovascular disease (Sorensen et al., 2003). PM exposure is also associated with elevated levels of CRP (Peters et al., 2001; Pope et al., 2004), enhanced production of proinflammatory cytokines (Ghio and Devlin, 2001; Seaton et al., 1999; Schwartz, 2001; Peters et al., 2001) and increased blood viscosity (Seaton et al., 1995; Peters et al., 1997), in epidemiological studies. In animal studies, the association between PM exposure and increased ET-1 has also been reported (Bouthillier et al., 1998; Vincent et al., 2001). It appears that PM and diabetes share common pathways in the development of cardiovascular diseases. Thus, we hypothesize that exposure to PM may increase the risk of cardiovascular diseases of diabetes through the enhanced production of oxidative stress, systemic inflammation, and endothelial dysfunction.

To determine whether PM exposure may induce synergistic effects in diabetics, we exposed streptozotocin (STZ)-induced diabetic rats to PM. STZ is a metabolite of the soil organism *Streptomyces achromogenes* and was first reported to be diabetogenic in studies of dogs and rats in 1963 (Bell and Hye, 1983). Diabetes is caused by a direct toxic effect of STZ on the pancreatic beta cell. After the administration of STZ, there is a characteristic increase in blood glucose, which is maintained at the level of 400 mg/dL or greater. This diabetic animal model has been used in many studies of diabetes pathophysiology for years (Vural et al., 2002; Ryu et al., 2003; Zhang et al., 2003).

2. Materials and methods

2.1. Collection and characterization of particles

The ambient particles in Hsin-Chuang, Taipei were collected by a particle concentrator (Sioutas et al., 1999). The particle concentrator used virtual impactor technology in which 110 L/min flow was channeled through a saturator, cooler, impactor, and diffusion dryer to generate concentrated particles with aerodynamic diameters between 0.01 and 2.5 μm. Particles were collected onto Teflon filters and the elemental compositions of the particles were determined using X-ray fluorescence (XRF, Model 6600; Jordan Valley AR, Inc., Migdal Haemek, Israel). In addition, data of water-soluble ions and carbonaceous content of $PM_{2.5}$ were obtained from Taiwan EPA supersite located at the sampling site. For these measurements, an R&P ambient carbon particulate monitor 5400 (Rupprecht & Patashnick Co., Inc., Albany, NY, USA) was used to determine the concentrations of organic and elemental carbons in $PM_{2.5}$. Meanwhile, an R&P ambient particulate nitrate monitor 8400N and an R&P ambient particulate sulfate monitor 8400S were used to determine the concentrations of $PM_{2.5}$ nitrate and sulfate. The characteristics of particles are listed in Table 1. The particulate properties represented typical urban, traffic-oriented ambient particles. All collected Teflon filters were equilibrated in $50 \pm 5\%$ relative humidity for more than 48 h and weighed before and after air sampling to obtain particle mass. After XRF analysis, six filters were submerged in endotoxin-free water and sonicated for 30 min to collect particles. These particles were pooled together before the instillation. Thus, exposed animals received the same components of particles.

2.2. Diabetic animals and intratracheal instillation of PM

Male Sprague–Dawley rats, weighing 200–250 g, were obtained from the National Laboratory Animal Breeding and Research Center, Taiwan. They were

Table 1
Characterization of fine particles instilled

Component	Concentration
Elemental components from XRF analysis	
Potassium	8.0%
Sulfur	1.5%
Aluminum	1.5%
Iron	0.9%
Phosphate	0.9%
Calcium	0.5%
Silicon	0.7%
Zinc	0.3%
Tungsten	0.04%
Vanadium	0.01%
Manganese	0.01%
Components of $PM_{2.5}$ ($\mu g/m^3$) from EPA supersite	
Organic carbon	9.8 (2.4)[a]
Elemental carbon	3.6 (3.2)
Sulfate	4.8 (1.2)
Nitrate	6.3 (3.4)

[a]Mean (SD).

housed in plastic cages on Aspen chip bedding and provided with Lab Diet 5001 and water ad lib, except during the exposure. Animals were maintained on a 12-h light/dark cycle at $22 \pm 1\,^{\circ}C$ and $55 \pm 10\%$ relative humidity. A single intraperitoneal (IP) injection of STZ (Sigma Chemical Co.; 60 mg/kg body weight, dissolved in citric acid buffer, pH 4.5) was administered to eight animals to induce diabetes (Bell and Hye, 1983), while the other eight rats were administered citric acid as nondiabetic rats. A dose of 200 μg particles was suspended in 0.5 mL of normal saline and sonicated for 20 min before use. The doses, which were expected to cause lung inflammation and possible subsequent systemic inflammation and endothelial dysfunction, were based on our experience and a previous study (Li et al., 1997). Eight rats ($N = 4$ for diabetic and nondiabetic rats, respectively) were anesthetized with pentobarbital (50 mg/kg body weight) and then instilled with PM intratracheally, while the other eight rats ($N = 4$ for diabetic and nondiabetic rats, respectively) were instilled with normal saline as controls. All protocols used in this experiment were approved by National Taiwan University's animal care and use committee.

2.3. Bronchoalveolar lavage (BAL) analysis

Rats were anesthetized with pentobarbital (50 mg/kg body weight) and sacrificed 24 h after intratracheal instillation. We chose 24 h because our previous experience indicated that significant pulmonary inflammation developed at 24 h after PM instillation, and it was hypothesized that systemic and endothelial inflammation developed following the lung inflammation. BAL fluid was collected by washing the airway with a phosphate-buffered saline (PBS) solution (pH 7.4, 28 mL/kg body weight) five times. Lavage fluid was used to determine the total number of cells and cell differential counts. Macrophages, lymphocytes, neutrophils, eosinophils, and basophils were counted under light microscopy (200 cells/slide). The remaining lavage fluid was used for the analysis of total protein and lactate dehydrogenase (LDH) activity. LDH activity was determined by autoanalyzer at National Taiwan University Hospital. Total protein was determined using total protein assay kit (Bio-Rad Co.)

2.4. Preparation of blood sample

A total of 15 mL whole blood was recovered from the aorta. Immediately, 1 mL of whole blood was collected in a citrate tube for blood glucose. Then 10 mL of whole blood was collected in ethylenediamine tetraacetic acid (EDTA) tubes. After being centrifuged at 1000*g* for 25 min, plasma aliquots were stored at $-80\,^{\circ}C$ for cytokine, 8-OHdG, ET-1, and [nitrate + nitrite] analyses. The remaining 4 mL whole blood was collected in a glass tube to clot for 2 h at room temperature. After being centrifuged for 20 min, serum samples were stored at $-80\,^{\circ}C$ for CRP analysis.

2.5. Determination of blood glucose

Blood glucose of the animals was determined using an autoanalyzer (Glucometer 3, blood glucose meter; Miles Inc.) at National Taiwan University Hospital.

2.6. Determination of plasma cytokine interleukin-6 (IL-6)

Rat IL-6 in plasma was determined using an enzyme-linked immunosorbent assay (ELISA) kit (R&D systems, Inc.). 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 rat IL-6 conjugate. Recombinant rat IL-6 ranging from 62.5 to 2000 pg/mL was used as standard. The minimum detectable concentration of rat IL-6 in this assay ranged from 14 to 36 pg/mL. All procedures followed the manufacturer's recommendation.

2.7. Determination of plasma cytokine tumor necrosis factor-α (TNF-α)

Rat TNF-α in plasma was determined using an ELISA kit (R&D systems, Inc.). A monoclonal antibody specific for rat TNF-α was coated on a 96-well polystyrene microplate. A polyclonal antibody against rat TNF-α conjugated to HRP was used as rat TNF-α

conjugate. Recombinant rat TNF-α ranging from 12.5 to 400 pg/mL was used as standard. The minimum detectable concentration of rat TNF-α in this assay was less than 5 pg/mL. All procedures followed the manufacturer's recommendation.

2.8. Determination of serum C-reactive protein

Rat serum CRP was determined using the CRP kit (Helica Biosystems, Inc.). An affinity-purified rabbit anti-rat CRP-IgG was precoated on a 96-well microplate. Concentrated affinity-purified HRP-labeled rabbit anti-rat CRP-IgG was used as conjugate. Rat serums ranging from 4.9 to 133.3 μg/mL were used as standards. The detection limit of this assay was 2.5 ng/mL. All procedures followed the manufacturer's recommendation.

2.9. Determination of plasma 8-OHdG

Ultrafiltered plasma samples were used for the determination of 8-OHdG levels with a competitive ELISA kit (OIS). The detection range was between 0.5 and 200 ng/mL. The 8-OHdG monoclonal antibody and plasma sample were loaded at 50 μL on a microtiter plate which had been coated with 8-OHdG. All procedures followed the manufacturer's recommendation.

2.10. Determination of plasma endothelin-1

Rat 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 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.

2.11. Determination of plasma [nitrate+nitrite]

The NO production was determined in plasma 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 μL of ultrafiltered plasma sample reacted with 1 mM NADPH and nitrate reductase for 60 min at room temperature. Then 10 μl 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.

2.12. Statistical analysis

The experimental design of this study is a 2^2 factorial design with four replicates for each treatment combination. The two factors are PM exposure and diabetes. The two levels for each factor are yes and no. Multiple response variables are measured simultaneously for each run. The 4 observations of each response variable in each treatment combination were first summarized by its mean and standard deviation to get an insight of the responses. We then used the Wilcoxon rank-sum test to examine the response difference between any two of the four treatment combinations because of only 4 observations in each treatment combination. Traditionally, the observations of a response variable in the factorial design are often fitted to a general linear model to estimate the main effects of the factors and interactions. Specifically, the 16 observations denoted by y_{ijk}, $i,j = 1,2$ and $k = 1,2,3,4$ of a response variable were further described by the linear statistical model $y_{ijk} = \mu + \alpha P_i + \beta D_j + \gamma PD_{ij} + \varepsilon_{ijk}$, where $P_i = 1$ when level i is the group exposed to PM and 0, otherwise; $D_j = 1$ when level j is the diabetes group and 0, otherwise; and $PD_{ij} = 1$ when $P_i = D_j$ and 0, otherwise. The error term is assumed to be independently and normally distributed. The parameter μ is the overall mean of the response variable. The coefficients, α, β, and γ in this model represent PM exposure effect, diabetes effect, and interaction between PM exposure and diabetes. We fit this general linear model and calculate the maximum likelihood estimates of these coefficients and their standard errors. Type I error was set at 0.05 for significance. The t statistic was used for testing significance of the coefficients. The residuals from individual fits were also examined. If diagnostic plots for the residuals show departures from the general linear models, we would find a proper transformation on the response variable or use Wilcoxon rank-sum tests instead for testing the two main effects with no testing for the interaction.

3. Results

3.1. Characteristics of study animals and effects of diabetes

Characteristics of study animals are described in Table 2. Body weight of diabetic rats was lower than that of nondiabetic rats (397.5 vs. 483.1 g). The mean plasma glucose level was 163 mg/dL in nondiabetic rats and 448.2 mg/dL in diabetic rats ($P < 0.05$).

Effects of diabetes on different parameters in BAL are described in Table 3. Diabetes had no effect on total cells, percentage of neutrophils, total protein, and LDH activity in BAL. In plasma analysis, diabetic rats demonstrated significantly greater 8-OHdG generation (6.2 vs. 6.8 ng/mL, $P<0.05$) and cytokine IL-6 (42.3 vs. 66.0 pg/mL, $P<0.05$) than nondiabetic rats. Furthermore, diabetic rats had significantly increased levels of plasma ET-1 (2.1 vs. 2.8 pg/mL, $P<0.05$) and decreased levels of plasma [nitrate + nitrite] (107.8 vs. 87.0 μM, $P<0.05$). There was no observable change in TNF-α and CRP in diabetic and nondiabetic rats.

3.2. Effects of PM exposure

In nondiabetic rats, PM caused significant increases in total cells and proportion of neutrophils in BAL (Table 3, $P<0.05$). Elevated total protein and LDH activity in BAL were also observed after PM exposure ($P<0.05$). Plasma 8-OHdG levels also showed an increase after PM exposure (Table 4, $P=0.08$). Similar findings were observed in plasma cytokine IL-6, TNF-α, and serum CRP ($P<0.05$). Furthermore, PM exposure caused a significant reduction of plasma [nitrate + nitrite] ($P<0.05$). However, there was no significant PM effect on plasma ET-1 in nondiabetic rats.

In STZ-diabetic rats, significant increases in pulmonary inflammation and injury markers were observed after PM exposure (Table 4, $P<0.05$). Plasma 8-OHdG and cytokine TNF-α significantly increased in diabetic rats after exposure to PM (Table 4, $P<0.05$). There were no significant alterations in cytokine IL-6 and CRP after PM exposure. In assessing endothelial function, we found a significant elevation of plasma ET-1 ($P<0.05$) and a decrease in plasma [nitrate + nitrite] ($P=0.08$) after exposure to PM.

3.3. Interaction between PM exposure and diabetes

When the PM effects were further compared in diabetic and nondiabetic rats, we found that increases of 8-OHdG and ET-1 were more prominent in diabetic rats. For 8-OHdG generation, diabetic rats exposed to PM demonstrated a 15.6% increase; however, nondiabetic rats exposed to PM showed only 4.0% increase. A 40.3% increase in plasma ET-1 after PM exposure was observed in diabetic rats, while there was only a 2.6% increase in plasma ET-1 after PM exposure in nondiabetic rats. The general linear model was further used to test the interaction between diabetes and PM. We found that there were interactions on 8-OHdG (Table 5, $P<0.01$) and ET-1 ($P=0.08$).

4. Discussion

In this study, we compared the influence of PM on parameters involved in cardiovascular disease in

Table 2
Characteristics of study animals

	Nondiabetic rats		STZ-diabetic rats	
	Saline	PM	Saline	PM
N	4	4	4	4
Body weight (g)	487.4 ± 29.5	476.7 ± 46.1	385 ± 37.8[a]	410 ± 25.8
Blood glucose (mg/dL)	166.7 ± 10.9	155.3 ± 10.7	467.7 ± 78.9[a]	435.5 ± 63.5[b]

Mean ± standard deviation.
[a] $P<0.05$ compared to nondiabetic rats with saline exposure.
[b] $P<0.05$ compared to STZ-diabetic rats with saline exposure.

Table 3
Bronchoalveolar lavage analyses in rats

	Nondiabetic rats		STZ-diabetic rats	
	Saline	PM	Saline	PM
N	4	4	4	4
Total number of cells ($\times 10^4$ cell)	60.4 ± 11.8	120.5 ± 12.5[a]	54.2 ± 10.2	136.5 ± 19.3[b]
Percentage of neutrophils	7.5 ± 3.2	43.7 ± 12.1[a]	9.2 ± 4.7	36.5 ± 2.4[b]
Total protein (mg/L)	400.7 ± 21.3	612.1 ± 37.5[a]	362.4 ± 50.8	620.5 ± 57.2[b]
LDH activity (U/L)	17.1 ± 13.2	81.2 ± 19.3[a]	22.5 ± 14.7	78.3 ± 20.4[b]

Mean ± standard deviation.
[a] $P<0.05$ compared to nondiabetic rats with saline exposure.
[b] $P<0.05$ compared to STZ-diabetic rats with saline exposure.

Table 4
Plasma and serum analysis of markers of oxidative stress, inflammation and endothelial function in rats

	Non-diabetic rats		STZ-diabetic rats	
	Saline	PM	Saline	PM
N	4	4	4	4
Oxidative stress				
Plasma 8-OHdG (ng/mL)	6.2±0.1	6.4±0.1[a]	6.8±0.1[b]	7.6±0.3[b,c]
Inflammation				
Serum CRP (μg/mL)	289.7±55.6	379.7±46.9[b]	355.5±48.9	381.1±30.2[b]
Plasma IL-6 (pg/mL)	42.3±6.2	71.6±0.3[b]	66.0±11.7[b]	90.3±29.5[b]
Plasma TNF-α (pg/mL)	7.7±0.8	9.7±0.8[b]	7.8±0.9	10.3±1.1[b,c]
Endothelial function				
Plasma ET-1 (pg/mL)	2.1±0.1	2.2±0.2	2.8±0.3[b]	3.2±0.1[b,c]
Plasma NO (μM)	107.8±10.4	89.5±2.8[b]	87.0±11.0[b]	71.5±3.8[b]

Mean±standard deviation.
[a] $P = 0.08$ compared to nondiabetic rats with saline exposure.
[b] $P < 0.05$ compared to nondiabetic rats with saline exposure.
[c] $P < 0.05$ compared to STZ-diabetic rats with saline exposure.

Table 5
Interaction between PM exposure and diabetes in plasma 8-OHdG and ET-1

	Plasma 8-OHdG			Plasma ET-1		
	Estimate value	Standard error	*P* value	Estimate value	Standard error	*P* value
Intercept	5.89	0.085	<0.01	1.92	0.092	<0.01
PM effect	0.49	0.085	<0.01	0.24	0.092	<0.05
Diabetes effect	0.91	0.085	<0.01	0.92	0.092	<0.01
Interaction between PM and diabetes	0.31	0.085	<0.01	0.17	0.092	0.08

diabetic and healthy rats. Our results showed that diabetic rats were associated with increased 8-OHdG, IL-6, and ET-1 but decreased [nitrate + nitrite]. In nondiabetic rats, PM exposure was also associated with increased plasma 8-OHdG, IL-6, TNF-α, and CRP but decreased [nitrate + nitrite]. Interestingly, we found that increases of 8-OHdG and ET-1 after PM exposure were more prominent in diabetic rats than in nondiabetic rats. Statistical analysis further indicated that there were interactions between diabetes and PM on plasma 8-OHdG and ET-1.

Diabetes mellitus is a highly prevalent chronic illness and affects approximately 100 million people worldwide (Amos et al., 1997). It has been proposed that endothelial cell inflammation, excessive ROS, and endothelial dysfunction may play important roles in diabetes-related cardiovascular diseases (Beckman et al., 2002). Our study has demonstrated that STZ-diabetic rats had significantly increased plasma ROS, cytokine IL-6, and ET-1 and decreased NO. We also found a borderline increase in serum CRP in STZ-diabetic rats. The results were consistent with those of previous studies (Ryu et al., 2003; Zhang et al., 2003; Pickup, 2004; Vural et al., 2002). Although diabetic rats were found to have endothelial dysfunction, we did not observe any lung inflammation or injury in diabetic rats without PM treatment. It appears that the STZ-diabetic rat is a sensitive model for studying cardiovascular diseases in diabetics.

In this study, we found significant increases in total cells, neutrophils, LDH, and protein in BAL after PM exposure in nondiabetic rats. These findings are consistent with those of previous studies (Gordon et al., 1998; Clarke et al., 1999; Lei et al., 2004). We also observed elevated PM-induced 8-OHdG and systemic inflammation, including IL-6, TNF-α, and CRP. The oxidative capacity of PM may be attributed to its transition metal constituents (Prahalad et al., 1999; Clarke et al., 2000). The concentration of transition metals on PM collected in our studies was much lower than that in other CRP studies conducted in the United States; therefore we speculate that the role of transition metals may be less significant on PM-related effects in this study. Interestingly, previous study has shown that ultrafine carbon black induces significantly higher ROS compared to fine carbon black under the same mass concentration (Wilson et al., 2002). Because our particles were collected from a particle concentrator

with size ranging between 0.01 and 2.5 μm, it is very likely that ultrafine particles account for, at least partly, the oxidative stress and inflammation. The elevations of oxidative stress may subsequently cause increases in IL-6 leading to lung inflammation (Tao et al., 2003). IL-6 has been reported to induce CRP in liver (Blake and Ridker, 2002). Recent studies have also demonstrated that CRP is an independent risk factor for cardiovascular diseases (Backes et al., 2004), and elevated CRP has been reported to be associated with PM exposure in elderly people (Pope et al., 2004). With regard to TNF-α, it is most likely secreted by alveolar macrophage after PM exposure (Becher et al., 2001). The effects of PM on lung inflammation and proinflammatory cytokines were consistent with those of previous studies.

NO exerts multiple modulating effects on inflammation and plays a key role in the regulation of vascular tone (Guzik et al., 2003). In our study, a significant decrease of nitrate and nitrite levels, an indicator of NO production, was observed in healthy rats after PM exposure. A recent study found a decrease in plasma NO level after instillation exposure to PM from Ottawa, although no statistical significance was reached (Ulrich et al., 2002). NO may be formed by inducible NO synthase (iNOS) in macrophages and other cells (Guzik et al., 2003). A previous study reported an increased production of iNOS-dependent NO by alveolar macrophage following dust exposure (Blackford et al., 1997). Although the NO decrease may have resulted from the PM effects in the airway, it is not clear whether the decreased NO also resulted from the PM effects on endothelium. NO may be formed from eNOS. It has been proposed that ROS may inhibit the activity of eNOS, which leads to reduction of NO synthesis (Beckman et al., 2002). It is plausible that the NO decrease is caused by ROS induced by penetrating PM (Nemmar et al., 2001, 2002) or indirectly by ROS formed in the lungs.

In our study, there was no increase for plasma ET-1 after PM instillation in nondiabetic rats. Data on the relationship between PM exposure and circulating ET-1 were limited. Recent studies reported an increase in plasma ET-1 after PM instillation in healthy rats, but they did not reach a significant level (Bouthillier et al., 1998; Ulrich et al., 2002). Another study proposed that concentrated ambient particle-induced vasoconstriction of small pulmonary arteries in rats might be due to elevated ET-1 production in pulmonary vessel endothelium (Batalha et al., 2002). Similar to NO reduction, PM may cause ET-1 increase directly through an effect on endothelium after penetrating into the circulation or indirectly through lung inflammation. It seems that PM with certain components and size may cause alterations in NO and ET-1.

In our study, we observed that PM and diabetic status individually altered oxidative stress and endothelial dysfunction, and the greatest effects were observed in diabetic rats with PM exposure. Significant synergistic interactions between PM exposure and diabetes on 8-OHdG and ET-1 levels were observed. In diabetes, hyperglycemia engenders adverse metabolic events with the generation of ROS in endothelial cells. We speculate that excessive ROS generation may affect the antioxidant capacity in endothelium and lead to further synergistic interaction on ROS generation after PM exposure. Subsequently, excessive ROS may impair endothelial function through increased ET-1. Evidence suggests a pathophysiologic role of ET-1 in diseases affecting the cardiovascular system, including hypertension, cerebrovascular disease, and heart failure (Schiffrin et al., 1997). In patients with heart failure, plasma ET-1 levels are two- to fourfold elevated and correlate with the severity of the disorder (Packer et al., 1993). Clinical observations also support a pathophysiologic role of ET-1 in coronary atherosclerosis. Increased plasma ET-1 concentrations were observed after myocardial infarction and persistent elevations of ET-1 predict an increased mortality within the subsequent 12 months (Lerman et al., 1991). In our earlier discussion, we have demonstrated that PM can increase the oxidative stress and cause endothelial dysfunction. Thus, it is plausible that PM exposure may enhance the risk of cardiovascular diseases in diabetes through the possible interaction between PM and diabetes in endothelium.

Plasma 8-OHdG was used as an indicator of oxidative stress in this study. Since 8-OHdG is an excision repair product of oxidative DNA damage, such damage in other tissues in addition to lung and endothelium may also contribute to the increase of plasma 8-OHdG. Previous study has shown that treatment of STZ resulted in elevated DNA damage in liver and kidney (Imaeda et al., 2002) and indirectly contributed to increased plasma 8-OHdG level. However, this did not affect the results in this study, because the PM effect on plasma 8-OHdG levels was compared in diabetic and nondiabetic rats. In addition, plasma 8-OHdG levels may also be affected by a decrease in clearance because of kidney disease in diabetics. Previous studies have reported increased glomerular filtration rate, decreased renal plasma flow, increased filtration fraction, and increased blood urea nitrogen in STZ-diabetic rats (Carney et al., 1979; Bell and Hye, 1983; Somova et al., 1988). Again, this did not affect the results in this study, because the PM effect on plasma 8-OHdG levels was compared in diabetic and nondiabetic rats.

Inflammation is also a crucial factor in cardiovascular disease and PM-induced effect. In this study, we did not observe significantly enhanced IL-6, TNF-α, and CRP in diabetic rats exposed to PM. Higher production of IL-6, TNF-α, and CRP have been reported in diabetic patients after PM exposure (Spranger et al., 2003).

However, why there is no interaction between diabetes and PM on inflammatory markers is not clear. One possible explanation is that cytokines have many different sources other than lung and endothelium (Rankin, 2004). Another possible explanation is that these cytokines are independently produced in lung and endothelium. However, the exact mechanisms need further study.

One limitation of this study is that instilled doses of particles were not adjusted by the body weight, although diabetic rats had lower body weight than nondiabetic rats. However, the PM effects in diabetic and nondiabetic rats are less likely to be affected, because the doses were proportionally higher in diabetic rats. Another limitation of this study is the small number of animals, although the statistical analysis has been properly conducted. Thus, further studies are needed to confirm our findings.

Our results revealed that PM and diabetic status individually altered oxidative stress and endothelial dysfunction, and the greatest effects were observed in diabetic rats with PM exposure. We conclude that PM exposure may enhance the risk of cardiovascular diseases through the possible interaction between PM and diabetes in endothelium. These findings provide further support for previous epidemiological studies. In this study, we also demonstrate that the STZ-diabetic rat is a useful model in studying PM-related cardiovascular effects.

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