



Exposure to ambient ultrafine particles and urinary 8-hydroxyl-2-deoxyguanosine in children with and without eczema

Sanghwan Song ^{a,*}, Domyung Paek ^b, Chunghee Park ^a, Chulwoo Lee ^a, Jung-Hyun Lee ^c, Seung-Do Yu ^a

^a Environmental Health Research Division, National Institute of Environmental Research, Incheon, Republic of Korea

^b Graduate School of Public Health, Seoul National University, Seoul, Republic of Korea

^c Samsung Medical Center, Seoul, Republic of Korea

HIGHLIGHTS

- Ambient ultrafine particles were associated with oxidative stress in children.
- Particles sized 47nm was most strongly associated with increase in urinary 8-OHdG.
- Immediate effect of particle on oxidative stress was observed in children with eczema.
- The effect of mass fraction particle on oxidative stress in children was less clear.

ARTICLE INFO

Article history:

Received 8 November 2012

Received in revised form 23 March 2013

Accepted 4 April 2013

Available online xxxx

Keywords:

Ultrafine particles

Polycyclic aromatic hydrocarbons

Oxidative stress

Children

Eczema

ABSTRACT

Ambient fine and ultrafine particles (UFPs) in urban air are known to contribute to inflammatory and allergic disease. It has been suggested that oxidative stress is an underlying mechanism for the detrimental health effects. The objective of this study was to investigate the short-term effect of ambient UFPs and particulate polycyclic aromatic hydrocarbons (PAHs) on urinary 8-hydroxyl-2-deoxyguanosine (8-OHdG) concentrations in children with and without eczema. Spot urine samples were collected from 84 children twice weekly for 61 days and 8-OHdG content was measured. Significant associations were found between the ambient UFPs and particle bound PAHs and increase in urinary 8-OHdG levels. An inter-quartile range (IQR) increase in the UFP concentration in the 24-h (IQR, 32,300/m³) period preceding urine collection was significantly associated with a 5.7% (95% confidence interval [CI], 0.16–1.27%) increase in the urinary 8-OHdG level children with AD. In children without eczema, such short-term effect of previous day UFPs on urinary 8-OHdG was not observed. There were no significant positive associations between the mass fraction of PMs and urinary 8-OHdG. The results suggest that short-term exposure to ambient UFPs plays a critical role in PM induced oxidative stress in children with eczema.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Ambient particulate matters (PMs) consist of particles of varying sizes originating from different sources. Epidemiological studies have linked a variety of adverse health effects, including respiratory and allergic diseases, to the presence of ambient PMs (Penttinen et al., 2001; Rodriguez et al., 2007; von Klot et al., 2002). However, the underlying mechanisms by which this occurs, and the properties of the particles responsible for the observed health effects, remain unclear.

Experimental studies have indicated that the ultrafine-sized fraction of diesel exhaust particles may increase allergic sensitization (Diaz-Sanchez et al., 1999, 2000). The small size relatively large surface area

per unit mass of ultrafine particles (UFPs) has prompted interest in their potential adjuvant effect (de Haar et al., 2006). Exposure to ambient UFPs is associated with decreased pulmonary function (Penttinen et al., 2001; Peters et al., 1997), increased asthma symptoms and medication use (von Klot et al., 2002), and an increased number of hospital admissions for pediatric asthma (Andersen et al., 2008). Previously, we and others examined the short term effect of ambient particles on symptoms in children with atopic dermatitis and found a positive association between UFPs and an exacerbation of eczema symptoms (Song et al., 2011). The biological effects of ambient UFPs appear to be linked to oxidative damage attributable to excessive ROS formation. Accumulating evidence suggests that these particles can penetrate tissues and induce the production of reactive oxygen species (ROS) (Li et al., 2003b; Oberdorster et al., 2005). The ROS not only aggravates airway inflammation by inducing pro-inflammatory mediators or enhancing bronchial hyper-responsiveness (Cho and Moon, 2010; Li et al., 2003a), but also may increase oxidative stress which is detected during acute

* Corresponding author at: Environmental Health Research Department, National Institute of Environmental Research, Ministry of Environment, Kyungseo-dong, Seo-gu, Incheon, Republic of Korea. Tel.: +82 32 560 7121; fax: +82 32 568 2035.
E-mail address: sanghwan@korea.kr (S. Song).

exacerbations of allergic symptom in the patients with atopic dermatitis (Tsukahara et al., 2003).

8-Hydroxyl-2-deoxyguanosine (8-OHdG) is a major by-product of hydroxyl radical attack on DNA, and is frequently measured in assessing oxidative damage. During the repair process that follows damage to DNA, 8-OHdG is excised and excreted into the urine without further metabolism (Marnett, 2000; Shigenaga et al., 1989). Urine collection is easy and non-invasive; thus urinary 8-OHdG is considered a convenient marker of oxidative stress (Loft et al., 1993; Pilger and Rudiger, 2006; Wu et al., 2004). Some clinical studies have measured urinary 8-OHdG to assess oxidative stress in patient with eczema (Nakai et al., 2009; Omata et al., 2001; Tsuboi et al., 1998). The association between ambient PM and urinary 8-OHdG levels has been inconsistent; a study have found that elevated levels of urinary 8-OHdG was associated with exposure to PM_{2.5} (less than 2.5 μm in diameter) in workplace (Kim et al., 2004) whereas, other failed to detect such association. These studies did not address responsible size mode of particles or modifying effect of allergic diseases on the oxidative damage.

The objectives of this study were to investigate the effect of exposure to urban ambient fine and ultrafine particles, as well as particulate PAHs, on oxidative stress in children and to examine whether the effect of particulate pollutants on oxidative stress was modified by the presence eczema. It was hypothesized that elevated concentrations of ambient UFPs are associated with an increase in urinary 8-OHdG, especially in children with eczema. Furthermore, this study examined whether urban particles of differing size modes may affect the oxidative damage differently. We performed a panel study that included repeated measurements of ambient pollutants and urinary 8-OHdG in children with and without eczema to assess transient within-subject variation in oxidative stress in response to changes in particulate pollutants. Understanding the effect of different particle sizes on induction of oxidative stress will be helpful in assessing the role of PM in inflammation process driving acute allergic exacerbation.

2. Material and methods

2.1. Study population

The study participants were 84 schoolchildren attending the same elementary school located in Incheon City, South Korea. We followed up all subjects to collect their urine two times a week for 61 days from April to June 2009, excluding holidays. The children were aged 8–12 years and were selected from 779 students in the school after preliminary examination to identify signs of eczema according to the Hanifin and Rajka's diagnostic criteria (Hanifin and Rajka, 1980). Forty one children were suffered from mild or intermediate eczema with severity scoring of atopic dermatitis or SCORAD indices (Stalder and Taieb, 1993) of 15–40. Forty three healthy, age- and sex-matched subjects were randomly assigned to a control group. Relevant information on individual characteristics such as passive smoking was obtained through a questionnaire completed by the children's parents. The study protocol was approved by an ethical committee in the National Institute of Environmental Research, and written consent was obtained from the parents of all study participants.

2.2. Urine collection

During the study, we visited the school approximately every 3 days to collect urine samples from the participants. Spot urine samples were obtained from each subject attending class, between 9:30 a.m. and noon on each sampling day. Up to 18 samples were collected for each subject. All urine samples were centrifuged at 2500 r/min for 10 min to eliminate suspended debris, and were then stored at $-20\text{ }^{\circ}\text{C}$ until further analysis.

2.3. Urinary 8-OHdG

Urinary 8-OHdG levels were determined using a competitive enzyme-linked immunosorbent assay kit (ELISA; Japan Institute for the Control of Aging, Fukuroi, Japan) following the manufacturer's instructions as described elsewhere (Kim et al., 2004; Lai et al., 2005; Wei et al., 2009). Briefly, 50- μL samples or standards were added to microtiter plates pre-coated with 8-OHdG, followed by 50- μL of primary anti-8-OHdG monoclonal antibody. The plates were then incubated at $37\text{ }^{\circ}\text{C}$ for 1 h. After washing, 100- μL of horseradish peroxidase-conjugated secondary antibody was added and incubated at $37\text{ }^{\circ}\text{C}$ for 1 h. Plates were washed again and 100- μL of chromogenic substrate was added and allowed to react at room temperature. Phosphoric acid was added to terminate the reaction. Absorbance at 450 nm was measured with a microplate spectrophotometer, and the limit of detection (LOD) was 0.2 ng/mL 8-OHdG. The concentration of 8-OHdG in each urine sample was adjusted to the creatinine concentration and was expressed as $\mu\text{g/g}$ creatinine. Urinary creatinine concentrations were determined by Jaffe's reaction using an automated analyzer (Hitachi 7600).

2.4. Ambient air pollutants

The ambient particles were measured continuously on the roof of the school during the period from March 27 to June 5 2009, as described previously (Song et al., 2012). All study subjects lived within a 2 km radius of the measurement site, which is situated close to a main road and 0.3 km from a 4-lane highway. Number concentrations (NC) of particles were measured with a scanning mobility particle sizer and counter (SMPS + C) in 7-min intervals. The SMPS + C consists of a differential mobility analyzer (DMA, Model 5.5-900 Grimm Aerosol Technik; GmbH & Co., Germany) and a condensation particle counter (CPC, Grimm Model 5.400), which counts the number of particles with a diameters between 11.1 and 1083.3 nm, classified in 44 channels. For analyses, 2 size fractions of 11.1–101 nm ($\text{NC}_{0.01-0.1}$), and 111–454 nm ($\text{NC}_{0.1-0.5}$) were calculated by combining the relevant channels. The mass concentrations of PM₁₀, PM_{2.5}, and PM₁ were measured by a multi-channel aerosol spectrometer (Grimm Model 1.107), which samples particles at 1.2 L/min using a laser light scattering optical particle counter. Particle-bound PAHs were measured with a photoelectric aerosol sensor direct reading instrument (PAS 2000, EcoChem Technologies) that detects PAH on particles by photoionization.

2.5. Data analysis

All statistical analyses performed using SAS software (version 9.1.3; SAS Institute, Cary, NC). As multiple samples were obtained from each subject, we used a linear mixed model (Proc mixed) to assess the association between air pollutants and 8-OHdG. The distributions of the urinary biomarker were skewed, and therefore, we log-transformed the data for analysis. Exposure variables were evaluated using the continuously monitored pollutants averaged over the 24 h (9 a.m. to 9 a.m.) preceding the time of urine collection (lag 1) as well as the 2 previous 24 h period (lags 2–3). To account for correlation among the repeated measurements per student, each models included random intercepts for each subject. A compound symmetric covariance structure was used to model repeated measures for each subject over time because it resulted in the best Akaike's Information Criterion compared with other covariance structures, including a first order autoregressive structure.

The effects of PMs and particle-bound PAHs on urinary 8-OHdG levels were estimated after adjusting for individual variables such as age, BMI, passive smoking, temperature on the previous day and time trend (sampling date). All effect estimates were expressed as percentage changes in urinary 8-OHdG associated with an increase of one inter-quartile range (IQR) for each air pollutant. In addition,

Table 1

Characteristics of study subjects and their urinary 8-OHdG concentration (mean $\pm$ SD or number).

	Children with eczema	Children without eczema
N	41	43
Age (years)	10 $\pm$ 1.3	10 $\pm$ 1.1
Male/female	24/17	24/19
Height (cm)	140.3 $\pm$ 9.6	138.6 $\pm$ 8.1
Weight (kg)	40 $\pm$ 12.6	36.5 $\pm$ 10.1
BMI (kg/m ²)	19.3 $\pm$ 4.4	18.7 $\pm$ 3.4
Live with smoker (n)	23	21
SCORAD index	15.3 $\pm$ 6.9	0
Urinary 8-OHdG (μ g/g crea.)	12.0 $\pm$ 6.3	10.1 $\pm$ 5.5

BMI, body mass index, weight (kg) $\div$ height (cm)² $\times$ 0.0001.

SCORAD index, scoring atopic dermatitis index.

we performed regression analyses on all subjects including interaction term between eczema status (dummy variable for eczema 1 and no eczema 0) and continuous exposure variable to test whether eczema was an effect modifier.

3. Results

Table 1 presents the basic characteristics of the study subjects. 84 subjects, 41 with eczema and 43 healthy children, with an average age of 10 years and ranged from 8 to 12 years, were included in the final analysis. None was diagnosed as having asthma or other respiratory diseases by a physician. 44 children (23 for eczema children) lived with a smoker. There were no differences in age, sex-ratio, and body mass index (BMI) between children with and without eczema. In total, 1269 (638 for atopic children) urine samples were obtained from the 84 subjects, with each individual providing 10–18 samples. The average urine 8-OHdG concentration for the atopic children was 12 μ g/g creatinine which was slightly higher than that in children without eczema.

Table 2 summarizes the concentrations of ambient particulate pollutant. The highest particle number was observed in the ultrafine fraction ranging from 0.01 to 0.1 μ m. Because the particles sized <500 nm constituted 96% of the total particle number concentration, the 33 channels of that particle size class (11.1–454 nm) were included in the analyses.

The 24-h averages ranged from 22.1 to 122.2 μ g/m³ for PM₁₀, 20.1 to 106.6 μ g/m³ for PM_{2.5}, and 8.7 to 34.0 ng/m³ for particle-bound PAHs. The 24-h averaged number concentrations of ambient particles were 9710 to 117,300 count/cm³ for NC_{0.01–0.1}, and 1688 to 15,480 count/cm³ for NC_{0.1–0.5} respectively.

Table 2

Summary of pollutants concentration 1–2 and 3-days prior to urine sample collection of 84 study subjects.

Pollutants		Lag 1	Lag 2	Lag 3	3-d mean
PM ₁₀ ^a	Mean $\pm$ SD	68.95 $\pm$ 25.52	69.43 $\pm$ 26.44	64.85 $\pm$ 26.77	66.78 $\pm$ 15.62
(μ g/m ³)	n	17	18	18	17
PM _{2.5}	Mean $\pm$ SD	55.15 $\pm$ 21.62	54.38 $\pm$ 22.43	49.46 $\pm$ 21.27	52.02 $\pm$ 12.79
(μ g/m ³)	n	17	18	18	17
PM ₁	Mean $\pm$ SD	48.64 $\pm$ 19.61	47.54 $\pm$ 21.19	42.62 $\pm$ 19.54	45.24 $\pm$ 11.75
(μ g/m ³)	n	17	18	18	17
NC _{0.1–0.5} ^b	Mean $\pm$ SD	7.48 $\pm$ 4	6.23 $\pm$ 3.3	6.21 $\pm$ 3	7.03 $\pm$ 2.54
(1000/cm ³)	n	17	16	15	14
NC _{0.01–0.1}	Mean $\pm$ SD	48.61 $\pm$ 30.73	38.69 $\pm$ 21.71	44.11 $\pm$ 29.58	46.26 $\pm$ 23.54
(1000/cm ³)	n	17	16	15	14
P-PAHs ^c	Mean $\pm$ SD	18.71 $\pm$ 6.69	15.34 $\pm$ 5.83	16.88 $\pm$ 8.29	17.31 $\pm$ 5.1
(ng/m ³)	n	17	17	16	16

Lag 1: 24 hour (09:00–09:00) average, lag 2: 25th to 48th hour average, lag 3: 49th to 72th hour average before urine sampling.

^a PM, particulate matter (numbers represent diameter of particles in μ m).

^b NC, number concentration of particles (numbers represent diameter of particles in μ m).

^c P-PAHs, particle-bound polycyclic aromatic hydrocarbons.

Table 3 shows the associations between the ambient particulate pollutants and urinary 8-OHdG levels in the study subjects. The mass fractions of PMs were not significantly associated with increased urinary 8-OHdG in both eczema and healthy children. There were inverse associations between the mass fraction of PMs (PM₁₀, PM_{2.5}, and PM₁) at lag 2 and urinary 8-OHdG concentrations in the atopic subjects, but these were not statistically significant except PM_{2.5}. On the contrary, the number concentration of ultrafine-sized particles measured at lags 1 to 2 days showed significant associations with increased in the urinary 8-OHdG concentrations in children with eczema. For an IQR increase in the NC_{0.01–0.1} concentrations at lag 1 and lag 2, the estimates of urinary 8-OHdG increase were 5.7% (95% CI, 1.3–10.2) and 6.6% (95% CI, 0.1–13.5), respectively. The particle-bound PAHs also showed significant and positive associations with urinary 8-OHdG concentrations in children with eczema. The largest effect was observed for the particle-bound PAHs at lag 1, which was associated with a urinary 8-OHdG increase of 14.4 (95% CI, 5.6–23.8).

There were significant associations between lagged 2-day number concentrations of particles and urinary 8-OHdG among subjects without eczema. For an IQR increase in the NC_{0.01–0.1} at lag 2, the estimates of urinary 8-OHdG increase were 13.4% (95% CI, 4.7–22.7) and 12.7% (95% CI, 6.5–19.2), respectively. However, NC_{0.01–0.1} was not significantly associated with the increase in urinary 8-OHdG at lag 1 day in children without eczema. The particle-bound PAHs also showed positive associations with urinary 8-OHdG concentrations in children without eczema. For an IQR increase in the particle-bound PAHs at lag 2 days, the estimates of urinary 8-OHdG increase were 16.8 (95% CI, 6.5–28.1). The urinary 8-OHdG changes per IQR change in each pollutant exposure were not significantly different between children with and without eczema (p-value for interaction > 0.05).

In analyzing the associations between the urinary 8-OHdG of children with eczema and the particle size classes sampled in the previous 24 h, we noted that the 8-OHdG increase was associated with the specific size ranges of ambient UFPs (Fig. 1). In previous 24 h, the urinary 8-OHdG was positively and significantly associated with particles in sizes ranging from 11.1 to 82.8 nm and 153 to 214 nm, and was most strongly associated with particles 46.8 nm in diameter (p = 0.003).

4. Discussion

We examined the association between ambient ultrafine-sized particles and oxidative DNA damage, as indicated by urinary 8-OHdG concentrations, in a panel of schoolchildren with and without eczema. We found that the ambient number concentration of particles, especially UFPs were significantly associated with urinary 8-OHdG concentrations.

Table 3Percentage changes^a in urinary 8-OHdG for interquartile range increase of ambient pollutants in linear mixed models.

Pollutants	IQR ^b	Changes of urinary 8-OHdG (%)		p-Value for interaction ^c
		Children with eczema	Children without eczema	
<i>PM₁₀</i> (μg/m ³)				
Lag 1	37.19	2.04 (−3.40 to 7.77)	−1.92 (−7.20 to 3.72)	0.26
Lag 2	37.19	−5.14 (−10.02 to −0.01)	0.15 (−4.97 to 5.54)	0.14
Lag 3	38.08	−1.29 (−7.12 to 4.92)	4.82 (−1.29 to 11.29)	0.11
<i>PM_{2.5}</i> (μg/m ³)				
Lag 1	33.01	3.23 (−2.70 to 9.52)	−0.26(−6.11 to 5.97)	0.41
Lag 2	32.65	−5.28 (−10.21 to −0.07)*	−0.52 (−5.80 to 5.03)	0.22
Lag 3	32.39	−1.89 (−7.81 to 4.42)	4.63 (−1.64 to 11.30)	0.07
<i>PM₁</i> (μg/m ³)				
Lag 1	31.84	4.51 (−1.83 to 11.26)	0.91 (−5.36 to 7.58)	0.40
Lag 2	31.21	−4.48 (−9.50 to 0.79)	−0.06 (−5.49 to 5.67)	0.30
Lag 3	31.46	−3.58 (−9.78 to 3.06)	3.73 (−2.91 to 10.81)	0.07
<i>NC_{0.1–0.5}</i> (1000/cm ³)				
Lag 1	5.49	5.96(0.15 to 12.10)*	−0.92 (−7.02 to 5.58)	0.22
Lag 2	5.32	4.11 (−2.68 to 11.38)	8.14 (1.13 to 15.63)*	0.19
Lag 3	5.51	1.38 (−8.23 to 12.00)	11.32 (0.58 to 23.20)*	0.16
<i>NC_{0.01–0.1}</i> (1000/cm ³)				
Lag 1	32.30	5.65 (1.31 to 10.18)**	1.99 (−2.93 to 7.16)	0.50
Lag 2	32.29	6.62 (0.12 to 13.54)*	13.37 (4.74 to 22.71)**	0.12
Lag 3	32.30	2.77 (−2.24 to 8.02)	5.87 (−3.71 to 16.41)	0.38
<i>P-PAHs^d</i> (ng/m ³)				
Lag 1	12.32	14.35 (5.60 to 23.83)**	12.63 (3.81 to 22.20)**	0.80
Lag 2	11.93	10.55 (0.60 to 21.47)*	16.82 (6.52 to 28.11)**	0.24
Lag 3	11.93	4.09 (−3.62 to 12.41)	7.29 (−0.67 to 15.89)	0.24

^a The percentage changes and 95% confidence intervals (CIs) were calculated as $[\exp(\text{model estimate} \times \text{IQR}) - 1] \times 100$, adjusted by age, sex, BMI, and passive smoke, date, temperature on previous day.

^b IQR: interquartile range (25–75 percentile).

^c Interaction product term: pollutants $\times$ eczema status (eczema, 1; no eczema, 0).

^d Particle-bound polycyclic aromatic hydrocarbons.

* <0.05.

** <0.01.

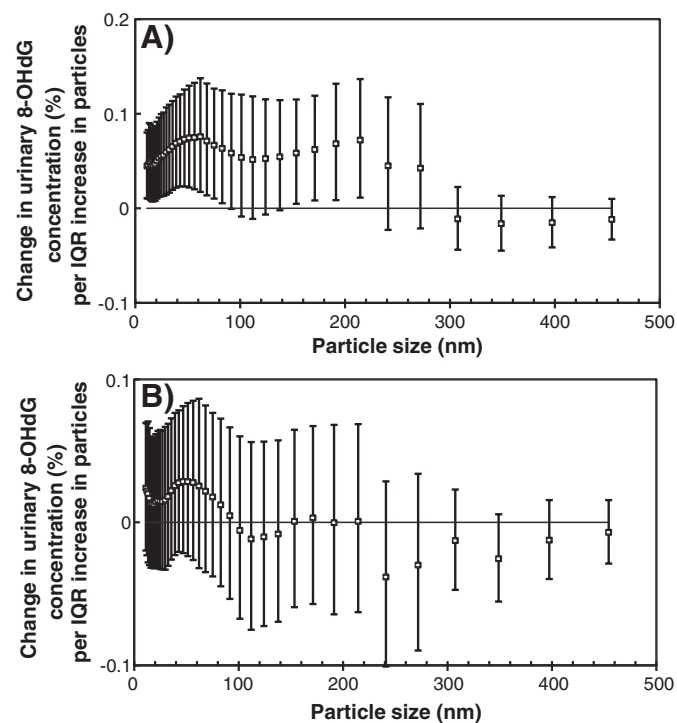


Fig. 1. Estimated percent changes of urinary 8-OHdG and 95% confidence intervals (vertical error bars) in children with atopic dermatitis (A) and without atopic dermatitis (B) by interquartile increase of particle number concentrations in 33 size classes (lag 1) after adjusting for age, sex, BMI, passive smoking, temperature and date.

UFPs are thought to be more toxic than larger particles (Donaldson et al., 2002; Li et al., 2003b) because of their small size, large surface area, and high content of redox-cycling chemicals, such as PAHs and quinines, which contribute to inflammation and oxidative stress (Li et al., 2008; Nel et al., 2006). Their unique small size may enable the UFP access to mitochondria and then release redox cycling chemicals that damage the inner membrane (Li et al., 2003b). ROS could be generated from ambient particles in several ways: the particles contain soluble transition metals that may generate ROS through Fenton-type reactions, the particle itself could induce phagocyte activation where ROS are released, and their chemical contents could activate quinone radicals generate ROS through redox cycling (Bolton et al., 2000; Li et al., 2003a; Sorensen et al., 2003b). These data are in accordance with previous *in vitro* and *in vivo* studies where nano-sized particles or UFPs have been shown to induce greater ROS and inflammatory responses (Brown et al., 2001; Li et al., 2003b; Oberdorster et al., 2005; Zhou et al., 2003). This result is also consistent with a recent controlled-exposure study in humans, in which exposure to UFPs, especially the 57-nm diameter fraction, showed significant association with oxidative stress markers (Brauner et al., 2007).

In this study, a significant association was observed between increased urinary 8-OHdG and ambient particle-bound PAHs, the metabolites of which can generate ROS in macrophages. Particulate PAHs have been known as a surrogate for other redox cycling chemicals being capable of inducing ROS production. This finding indicates that it is not only the size of the particles but also the compounds associated with them that are important factors in particle-induced oxidative stress.

Our results indicated that the eczema status did not significantly modify the associations between oxidative damage and particulate pollutants. However, the children with eczema seem to have a faster response to UFP exposure than children without eczema. We found that

the previous 24-h averaged concentrations of UFPs were associated with increases in urinary 8-OHdG in children with eczema, with the strongest association observed for the particle size of 47 nm. Such short-term effects imply that exposure to ambient UFPs might have a rapid affect on eczema patients to increase oxidative stress. Atopic eczema, a chronic inflammation of the skin is mediated by increased activated T cells, eosinophils, macrophages, and IgE (Beck and Leung, 2000; Spergel et al., 1998) that also produce increased amounts of ROS after immunological stimulation (Leung, 1999; Okayama, 2005; Tsuboi et al., 1998). Previous studies showed that urinary 8-OHdG levels in patients with atopic eczema were significantly higher than in healthy controls, suggesting that bioactive substances such as cytokines, chemokines, and ROS are released during inflammation of the skin (Nakai et al., 2009; Omata et al., 2001). It seems that such changes could create a vicious cycle leading to further immune activation, oxidative stress, and thus propagation of skin inflammation (Omata et al., 2001). Our data supported the hypothesis that the ambient UFP can act as adjuvants for allergic sensitization, probably due to their high oxidative potential (de Haar et al., 2006; Li et al., 2009).

We observed no significant effect on urinary 8-OHdG of exposure to the mass fraction of PMs. However, a few studies have indicated that PM₁₀ and PM_{2.5} could induce oxidative DNA damage in individuals exposed to high concentrations such as those that might occur in the workplace (Kim et al., 2004; Wei et al., 2009), or among residents living near a desert (Bae et al., 2010). Sorensen et al. (2003a) also found that personal exposure to PM_{2.5} was associated with oxidative DNA damage in schoolchildren, but not with urinary 7-hydro-8-oxo-2'-deoxyguanosine (8-oxodG) levels.

To our knowledge, this is the first panel study for children with eczema to examine the association between the size of ambient particulate pollutants and an oxidative stress biomarker. The strength of this study is that we linked the different sizes of ambient particles to the change in concentration of the urinary oxidative DNA damage marker. Of note is that we also assessed the particle size classes to identify the most relevant particle size to the increased oxidative DNA damage marker.

Some limitations of the study should be considered in interpreting our results. First, although we measured urinary 8-OHdG to assess the magnitude of oxidative stress, the significance of urinary 8-OHdG is not fully understood (Pilger and Rudiger, 2006). For example, the excretion of 8-OHdG in urine may depend on other factors, such as DNA repair capacity and confounding variables such as diet, physical activity, or the vitamin status (Bianchini et al., 2001; Chen et al., 1999; Okamura et al., 1997) that we did not measure. Second, the particle measurements were performed at a fixed monitoring site; this is unlikely to reflect the exposure level of children who lived far from the school, and may thus result in a misclassification of the exposure. Third, we studied only 84 children who were subjected to repeated measurements over a relatively short period (2 months). While the short period is a limitation, the use of repeated measurements increased the power of the study allowing us to examine the association between measured ambient particles and the urinary marker.

In conclusion, this panel study of schoolchildren shows that urinary 8-OHdG is positively associated with short-term exposure to ambient UFPs but not with the mass fraction of PMs. Our results suggest an acute effect of an increased UFP on increase in urinary 8-OHdG in children with eczema. Especially, the particles less than 50 nm play a critical role in PM induced the oxidative stress. Thus, the ambient particles derived mainly from traffic sources, such as diesel exhaust particles, as well as PAHs, may rapidly enhance oxidative stress in children with pre-existing eczema living in urban areas.

Conflict of interest

There is no conflict of interest.

Acknowledgments

This work was supported by the National Institute of Environmental Research (2009), Republic of Korea. The study protocol was approved by an ethical committee in the National Institute of Environmental Research.

References

- Andersen ZJ, Wahlin P, Raaschou-Nielsen O, Ketzel M, Scheike T, Loft S. Size distribution and total number concentration of ultrafine and accumulation mode particles and hospital admissions in children and the elderly in Copenhagen, Denmark. *Occup Environ Med* 2008;65:458–66.
- Bae S, Pan XC, Kim SY, Park K, Kim YH, Kim H, et al. Exposures to particulate matter and polycyclic aromatic hydrocarbons and oxidative stress in schoolchildren. *Environ Health Perspect* 2010;118:579–83.
- Beck LA, Leung DY. Allergen sensitization through the skin induces systemic allergic responses. *J Allergy Clin Immunol* 2000;106:S258–63.
- Bianchini F, Jaekel A, Vineis P, Martinez-Garcia C, Elmstahl S, van Kappel AL, et al. Inverse correlation between alcohol consumption and lymphocyte levels of 8-hydroxydeoxyguanosine in humans. *Carcinogenesis* 2001;22:885–90.
- Bolton JL, Trush MA, Penning TM, Dryhurst G, Monks TJ. Role of quinones in toxicology. *Chem Res Toxicol* 2000;13:135–60.
- Brauner EV, Forchhammer L, Moller P, Simonsen J, Glasius M, Wahlin P, et al. Exposure to ultrafine particles from ambient air and oxidative stress-induced DNA damage. *Environ Health Perspect* 2007;115:1177–82.
- Brown DM, Wilson MR, MacNee W, Stone V, Donaldson K. Size-dependent proinflammatory effects of ultrafine polystyrene particles: a role for surface area and oxidative stress in the enhanced activity of ultrafines. *Toxicol Appl Pharmacol* 2001;175:191–9.
- Chen L, Bowen PE, Berzy D, Aryee F, Stacewicz-Sapuntzakis M, Riley RE. Diet modification affects DNA oxidative damage in healthy humans. *Free Radic Biol Med* 1999;26:695–703.
- Cho YS, Moon HB. The role of oxidative stress in the pathogenesis of asthma. *Allergy Asthma Immunol Res* 2010;2:183–7.
- de Haar C, Hassing I, Bol M, Bleumink R, Pieters R. Ultrafine but not fine particulate matter causes airway inflammation and allergic airway sensitization to co-administered antigen in mice. *Clin Exp Allergy* 2006;36:1469–79.
- Diaz-Sanchez D, Garcia MP, Wang M, Jyrala M, Saxon A. Nasal challenge with diesel exhaust particles can induce sensitization to a neoallergen in the human mucosa. *J Allergy Clin Immunol* 1999;104:1183–8.
- Diaz-Sanchez D, Penichet-Garcia M, Saxon A. Diesel exhaust particles directly induce activated mast cells to degranulate and increase histamine levels and symptom severity. *J Allergy Clin Immunol* 2000;106:1140–6.
- Donaldson K, Brown D, Clouter A, Duffin R, MacNee W, Renwick L, et al. The pulmonary toxicology of ultrafine particles. *J Aerosol Med* 2002;15:213–20.
- Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol Suppl (Stockh)* 1980;92:44–7.
- Kim JY, Mukherjee S, Ngo LC, Christiani DC. Urinary 8-hydroxy-2'-deoxyguanosine as a biomarker of oxidative DNA damage in workers exposed to fine particulates. *Environ Health Perspect* 2004;112:666–71.
- Lai CH, Liou SH, Lin HC, Shih TS, Tsai PJ, Chen JS, et al. Exposure to traffic exhausts and oxidative DNA damage. *Occup Environ Med* 2005;62:216–22.
- Leung DY. Pathogenesis of atopic dermatitis. *J Allergy Clin Immunol* 1999;104:S99–S108.
- Li N, Hao M, Phalen RF, Hinds WC, Nel AE. Particulate air pollutants and asthma. A paradigm for the role of oxidative stress in PM-induced adverse health effects. *Clin Immunol* 2003a;109:250–65.
- Li N, Sioutas C, Cho A, Schmitz D, Misra C, Sempf J, et al. Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage. *Environ Health Perspect* 2003b;111:455–60.
- Li N, Xia T, Nel AE. The role of oxidative stress in ambient particulate matter-induced lung diseases and its implications in the toxicity of engineered nanoparticles. *Free Radic Biol Med* 2008;44:1689–99.
- Li N, Wang M, Bramble LA, Schmitz DA, Schauer JJ, Sioutas C, et al. The adjuvant effect of ambient particulate matter is closely reflected by the particulate oxidant potential. *Environ Health Perspect* 2009;117:1116–23.
- Loft S, Fischer-Nielsen A, Jeding IB, Vistisen K, Poulsen HE. 8-Hydroxydeoxyguanosine as a urinary biomarker of oxidative DNA damage. *J Toxicol Environ Health* 1993;40:391–404.
- Marnett LJ. Oxyl radicals and DNA damage. *Carcinogenesis* 2000;21:361–70.
- Nakai K, Yoneda K, Maeda R, Munehiro A, Fujita N, Yokoi I, et al. Urinary biomarker of oxidative stress in patients with psoriasis vulgaris and atopic dermatitis. *J Eur Acad Dermatol Venereol* 2009;23:1405–8.
- Nel A, Xia T, Madler L, Li N. Toxic potential of materials at the nanolevel. *Science* 2006;311:622–7.
- Oberdorster G, Oberdorster E, Oberdorster J. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. *Environ Health Perspect* 2005;113:823–39.
- Okamura K, Doi T, Hamada K, Sakurai M, Yoshioka Y, Mitsuzono R, et al. Effect of repeated exercise on urinary 8-hydroxy-deoxyguanosine excretion in humans. *Free Radic Res* 1997;26:507–14.
- Okayama Y. Oxidative stress in allergic and inflammatory skin diseases. *Curr Drug Targets Inflamm Allergy* 2005;4:517–9.
- Omata N, Tsukahara H, Ito S, Ohshima Y, Yasutomi M, Yamada A, et al. Increased oxidative stress in childhood atopic dermatitis. *Life Sci* 2001;69:223–8.

- Penttinen P, Timonen KL, Tiittanen P, Mirmé A, Ruuskanen J, Pekkanen J. Number concentration and size of particles in urban air: effects on spirometric lung function in adult asthmatic subjects. *Environ Health Perspect* 2001;109:319–23.
- Peters A, Wichmann HE, Tuch T, Heinrich J, Heyder J. Respiratory effects are associated with the number of ultrafine particles. *Am J Respir Crit Care Med* 1997;155:1376–83.
- Pilger A, Rudiger HW. 8-Hydroxy-2'-deoxyguanosine as a marker of oxidative DNA damage related to occupational and environmental exposures. *Int Arch Occup Environ Health* 2006;80:1–15.
- Rodriguez C, Tonkin R, Heyworth J, Kusel M, De Klerk N, Sly PD, et al. The relationship between outdoor air quality and respiratory symptoms in young children. *Int J Environ Health Res* 2007;17:351–60.
- Shigenaga MK, Gimeno CJ, Ames BN. Urinary 8-hydroxy-2'-deoxyguanosine as a biological marker of *in vivo* oxidative DNA damage. *Proc Natl Acad Sci U S A* 1989;86:9697–701.
- Song S, Lee K, Lee YM, Lee JH, Lee SI, Yu SD, et al. Acute health effects of urban fine and ultrafine particles on children with atopic dermatitis. *Environ Res* 2011;111:394–9.
- Song S, Paek D, Lee Y-M, Lee C, Park C, Yu S-D. Ambient fine and ultrafine particle measurements and their correlations with particulate PAHs at an elementary school near a highway. *Asian J Atmos Environ* 2012;6:96–103.
- Sorensen M, Autrup H, Hertel O, Wallin H, Knudsen LE, Loft S. Personal exposure to PM_{2.5} and biomarkers of DNA damage. *Cancer Epidemiol Biomarkers Prev* 2003a;12:191–6.
- Sorensen M, Autrup H, Moller P, Hertel O, Jensen SS, Vinzents P, et al. Linking exposure to environmental pollutants with biological effects. *Mutat Res* 2003b;544:255–71.
- Spergel JM, Mizoguchi E, Brewer JP, Martin TR, Bhan AK, Geha RS. Epicutaneous sensitization with protein antigen induces localized allergic dermatitis and hyperresponsiveness to methacholine after single exposure to aerosolized antigen in mice. *J Clin Invest* 1998;101:1614–22.
- Stalder JF, Taieb A. Severity scoring of atopic dermatitis: The SCORAD index. *Dermatology* 1993;186:23–31.
- Tsuboi H, Kouda K, Takeuchi H, Takigawa M, Masamoto Y, Takeuchi M, et al. 8-Hydroxydeoxyguanosine in urine as an index of oxidative damage to DNA in the evaluation of atopic dermatitis. *Br J Dermatol* 1998;138:1033–5.
- Tsukahara H, Shibata R, Ohshima Y, Todoroki Y, Sato S, Ohta N, et al. Oxidative stress and altered antioxidant defenses in children with acute exacerbation of atopic dermatitis. *Life Sci* 2003;72:2509–16.
- von Klot S, Wolke G, Tuch T, Heinrich J, Dockery DW, Schwartz J, et al. Increased asthma medication use in association with ambient fine and ultrafine particles. *Eur Respir J* 2002;20:691–702.
- Wei Y, Han IK, Shao M, Hu M, Zhang OJ, Tang X. PM_{2.5} constituents and oxidative DNA damage in humans. *Environ Sci Technol* 2009;43:4757–62.
- Wu LL, Chiou CC, Chang PY, Wu JT. Urinary 8-OHdG: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetics. *Clin Chim Acta* 2004;339:1–9.
- Zhou YM, Zhong CY, Kennedy IM, Leppert VJ, Pinkerton KE. Oxidative stress and NFkappaB activation in the lungs of rats: a synergistic interaction between soot and iron particles. *Toxicol Appl Pharmacol* 2003;190:157–69.