Exposure to Benzo[a]pyrene and 1-Nitropyrene in Particulate Matter Increases Oxidative Stress in the Human Body

Sun-Haeng Choi , Bolormaa Ochirpurev , Akira Toriba , Jong-Uk Won , Heon Kim *

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Article

Exposure to Benzo[a]pyrene and 1-Nitropyrene in Particulate Matter Increases Oxidative Stress in the Human Body

Sun-Haeng Choi [1,2,†], Bolormaa Ochirpurev [3,†], Akira Toriba [4], Jong-Uk Won [2] and Heon Kim [1,3,*]

[1] Department of Occupational and Environmental Medicine, Chungbuk National University Hospital, Cheongju, Republic of Korea; shchoi@cbnuh.or.kr (S.-H.C.); kimheon@cbu.ac.kr (H.K.)
[2] Department of Public Health, Graduate School, Yonsei University, Seoul, Republic of Korea; juwon@yuhs.ac (J.-U.W.)
[3] Department of Preventive Medicine, College of Medicine, Chungbuk National University, Cheongju, Republic of Korea; bolormaa@chungbuk.ac.kr (B.O.)
[4] Department of Hygienic Chemistry, Graduate School of Biomedical Science, Nagasaki University, Nagasaki, 852-8521, Japan; toriba@nagasaki-u.ac.jp (A.T.)
* Correspondence: kimheon@cbu.ac.kr
† These authors contributed equally to the article as first authors.

Abstract: Polycyclic aromatic hydrocarbons (PAHs) have been reported to cause oxidative stress in metabolic processes. This study aimed to evaluate the relationship between exposure to PAHs, including benzo[a]pyrene (BaP) and 1-nitropyrene (1-NP), in the atmosphere and oxidative stress levels in the human body. This study included 44 Korean adults who lived in Cheongju, South Korea. Atmospheric BaP and 1-NP concentrations and urinary 6-hydroxy-1-nitropyrene (6-OHNP), N-acetyl-1-aminopyrene (1-NAAP), and 1-hydroxypyrene (1-OHP) concentrations were measured. The oxidative stress level was assessed by measuring urinary thiobarbituric acid reactive substances (TBARS) and 8-hydroxydeoxyguanosine (8-OHdG) concentrations. Urinary TBARS and 6-OHNP concentrations significantly differed between winter and summer. BaP exposure was significantly associated with urinary 8-OHdG concentrations in summer. However, atmospheric 1-NP did not show a significant correlation with oxidative stress marker concentrations. Urinary 1-NAAP concentration was a significant determinant for urinary 8-OHdG concentration in summer. Oxidative stress in the body increases in proportion to inhalation exposure to BaP, and more 8-OHdG is produced in the body as the amount of 1-NP, which is metabolized to 1-AP or 1-NAAP, increases.

Keywords: polycyclic aromatic hydrocarbons; benzo[a]pyrene; 1-nitropyrene; oxidative stress; thiobarbituric acid reactive substances; 8-hydroxydeoxyguanosine

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are organic compounds formed by the incomplete combustion or pyrolysis of organic substances, including tobacco and fossil fuels, and are composed of two or more fused benzene rings. Various types of PAHs exist in particulate matter (PM), a significant air pollutant. Benzo[a]pyrene (BaP) is a representative PAH, and it has been reported that environmental or occupational exposure to BaP showed adverse effects on neurobehavioral function in adults and on neurodevelopment in children [1, 2]. Moreover, the International Agency for Research on Cancer (IARC) classifies BaP as a human carcinogen [3]. BaP absorbed in the body induces cytochrome P450 1A1 activation and is metabolized to benzo[a]pyrene-7,8-diol-9,10-epoxide (BPDE) [4]. Excessive reactive oxygen species (ROS) are generated during this metabolic process [5].

Pyrene, one of the PAHs, is metabolized into 1-hydroxypyrene (1-OHP) by the cytochrome P450 enzyme and excreted into the urine. The 1-OHP urinary concentration is frequently used as an indicator of exposure levels to PAHs.

1-Nitropyrene (1-NP), one of the PAHs, is specifically produced during diesel fuel combustion and has been reported to cause genetic mutations and chromosomal aberration. The IARC classifies

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1-NP as a Group 2A chemical [3]. Exposure to 1-NP increases oxidative stress, inflammation, and endothelial dysfunction, thereby resulting in an increased risk of cardiovascular disease. To evaluate the exposure level to 1-NP, the concentration of 1-NP metabolites, including 6-hydroxy-1-nitropyrene (6-OHNP), 1-aminopyrene (1-AP), and N-acetyl-1-aminopyrene (1-NAAP), in the urine is measured.

BaP and 1-NP are believed to cause oxidative stress and harmful effects on the human body through these mechanisms; however, few studies have confirmed that ROS increases in the human body owing to BaP or 1-NP exposure.

PM concentration in Korea's atmosphere is higher than those of in developed countries, particularly in winter. Cheongju, a city located in the center of Korea, has a large industrial complex and three waste incinerators; the PM brought across the border by the northwest wind in winter makes this city an area with a high PM concentration in Korea.

This study aimed to evaluate the relationship between exposure to PAHs, such as BaP and 1-NP, in the atmosphere and oxidative stress levels in the body of individuals living in Cheongju.

2. Materials and Methods

2.1. Study participants

This study included 44 adults living around the industrial complex in Cheongju, a waste incinerator, or in suburban areas. Data on demographic factors, smoking habits, and daytime activity were collected using a questionnaire. They all provided the first urine in the morning of the day following atmospheric sampling. Participants received a thorough explanation of the study and provided written consent.

2.2. BaP and 1-NP Atmospheric Air Concentration Measurement

Atmospheric air was sampled using a personal air sampler (Apex Standard, SN0376420 Casella CEL, Bedford, UK) once in summer and once in winter. A 35-mm PTFE filter with a 2-μm pore size was attached to a personal air sampler and sampled for more than 24 h at a rate of approximately 3 L/min.

The filter was placed in a flask, 2 mL of dichloromethane was added, vortex mixed, and ultrasonically extracted. The extract was completely dried and re-dissolved in acetonitrile. Aliquots of the solution were subsequently injected into a two-dimensional high-performance liquid chromatography (HPLC) system with a fluorescence detector (FD) for the quantification of BaP and 1-NP, respectively [6]. The injected sample was eluted through a clean-up column (Cosmosil, 5NPE, 150 × 4.6 mm i.d., 5 μm, Nacalai Tesque, Kyoto, Japan) with its guard column 1 (10 × 4.6 mm i.d.); subsequently, 1-NP was reduced to 1-aminopyrene by using a reduction column (NPpak-RS, 10 × 4.6 mm i.d., Jasco, Tokyo, Japan) at 80°C. The mobile phase for the clean-up and reduction columns was ethanol/acetate buffer (pH, 5.5) (95/5, v/v) at a 0.2-mL/min flow rate. A fraction of 1-AP and unchanged BaP eluted from the reduction column with the mobile phase was mixed with 30-mM ascorbic acid at a 1.6-mL/min flow rate and subsequently trapped on the concentration column (Spheri-5 RP-18, 30 × 4.6 mm i.d., 5 μm, Perkin Elmer, MA, USA). The concentrated fraction was passed through two separation columns (Inertsil ODS-P, 250 × 4.6 mm i.d., 5 μm, GL Sciences, Tokyo, Japan) with their guard column (10 × 4.6 mm i.d.) in tandem. All columns except the reduction column were maintained at 20°C. A programmed gradient elution of the separation columns was performed using 10-mM imidazole buffer (pH, 7.6) as eluent A and acetonitrile as eluent B. Finally, the separated analytes were detected the dual-channel FD. The excitation/emission wavelengths were 260/420 and 254/425 nm for BaP and 1-AP, respectively.

2.3. 8-Hydroxydeoxyguanosine (8-OHdG) Urinary Concentration Measurement

The 8-OHdG urinary concentration was measured using an ELISA kit (New 8-OHdG Check, JaICA, Fukuroi, Japan) according to the manufacturer's instructions. A brief description of the measurement method is as follows. The urine samples and standards were added to the 8-OHdG–coated microtiter plate. After washing, an enzyme-labeled secondary antibody was added to the plate.

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Unbound HRP-conjugated secondary antibody was washed out, and the substrate solution was added. After the termination of the reaction, absorbance was read at 450 nm.

2.4. Thiobarbituric Acid Reactive Substance (TBARS) Urinary Concentration Measurement

TBARS were measured at three different wavelengths (fluorescence, λ-ex 530 nm and λ-ex 550 nm; λ-ex 515 nm and λ-ex 553 nm; and absorbance, 532 nm) using a microplate reader [7]. The TBARS concentration was estimated using the following equation: TBARS level (μM) = −0.282 + 1.830 × (the TBARS level measured at the fluorescence wavelengths of λ-ex 530 nm and λ-em 550 nm, μM) −0.685 × (the TBARS level measured at the fluorescence wavelengths of λ-ex 515 nm and λ-em 553 nm, μM) + 0.035 × (the TBARS level measured at the absorbance wavelength of 532 nm, μM).

2.5. 1-OHP Urinary Concentration Measurement

To 45 mL of urine, 4.5 mL of 1-M sodium acetate buffer (pH, 5.0), 450 μL of β-glucuronidase/aryl sulfatase (type H-2, from *Helix pomatia*: β-glucuronidase activity, 100,000 units/mL; and sulfatase activity, 7,500 units/mL), and 50 mg of blue rayon were added, and the mixtures were incubated at 37°C for 16 h. Blue rayon was removed, washed with water, and eluted with 2 mL of methanol and 2 mL of methanol + 1% NH_3 solution. After evaporating to dry with N_2 gas, it was dissolved again in 400-μL methanol. Fifty microliters of this eluent were used for 1-OHP concentration measurement, and the rest of the eluent was used for 6-OHNP and 1-NAAP concentration measurement.

The 1-OHP concentration was measured using an HPLC (LC-20AD, Shimadzu, Kyoto, Japan) with a 250 × 4.6 mm reverse phase column (J'sphere ODS-H80, YMC, Kyoto, Japan) and a fluorescent detector (RF-20A, Shimadzu, Kyoto, Japan). The mobile phase was 65% acetonitrile, and the flow rate was 1 mL/min. The excitation/emission wavelength of the FD was 242 nm/388 nm [8].

2.6. 6-OHNP and 1-NAAP Urinary Concentration Measurement

Nitroreduction reaction was performed by passing the remaining eluent through the online reduction column (NPpak-RS). 6-Hydroxy-1-aminopyrene concentrations were measured with the same HPLC system with column and mobile phase as those used for the measurement of 1-OHP concentration. The excitation/emission wavelength of the FD was 285 nm/428 nm.

Amide reaction was performed at room temperature for 1 h by adding 200 μL of acetic anhydride to the nitroreduced fraction. Since we used an amidification method of converting 1-AP to 1-NAAP, the 1-NAAP concentration measured in this study is the sum of the 1-AP and 1-NAAP concentrations. 1-NAAP concentration was measured using the same HPLC system with the column and mobile phase as those used for the measurement of 1-OHP concentration. The excitation/emission wavelength of the FD was 273 nm/385 nm [9].

The urinary concentrations of those substances were corrected by the urinary creatinine concentration.

2.7. Statistical Analysis

Paired T-tests were used to compare summer and winter measurements. Regression analysis was performed to determine the relationship between BaP and 1-NP exposure levels, their urinary metabolite concentrations, and urinary 8-OHdG and TBARS concentrations. A general linear model was used to statistically analyze the effects of BaP or 1-NP exposure on 8-OHdG and TBARS urinary concentrations after controlling for age, sex, smoking habit and the levels of the other exposure markers.

Statistical analysis was performed using SAS OnDemand (SAS Institute, Cary, NC, USA). A p-value of <0.05 was considered statistically significant.

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3. Results

The general characteristics of the study participants are shown in Table 1. The mean age was 63.8 years, and approximately 60% (26/44) of them were females. Current smokers were 16.0%. Residents who were living near the incinerator, those living near the industrial complex, and rural residents were 36.4%, 22.7%, and 40.9%, respectively.

Table 1. General characteristics of the study participants.

Variables		N (%)	Mean ± S.D.
Age		44 (100%)	63.8 ± 12.1 yr
Sex	Male	18 (40.9%)	
	Females	26 (59.1%)	
Smoking habit	Never smokers	28 (63.6%)	
	Ex-smokers	9 (20.5%)	
	Current smokers	7 (16.0%)	
Residential area	Incinerator area	16 (36.4%)	
	Industrial complex area	10 (22.7%)	
	Rural area	18 (40.9%)	

S.D.: standard deviation.

BaP and 1-NP atmospheric air concentrations and TBARS, 8-OHdG, 1-OHP, 6-OHNP, and 1-NAAP urinary concentrations are presented in Table 2. The concentrations of BaP and 1-NP in the atmospheric air were significantly higher in winter than those in summer. TBARS and 6-OHNP urinary concentrations were significantly different between winter and summer; however, 8-OHdG, 1-OHP and 1-NAAP urinary concentrations were not.

Table 2. Benzo[a]pyrene and 1-nitropyrene atmospheric air concentrations and TBARS, 8-hydroxydeoxyguanosine, 1-OHP, 6-OHNP, and 1-NAAP urinary concentrations.

Variables	Mean ± SD		p-value
	Winter (N = 44)	Summer (N = 44)	
Benzo[a]pyrene in the atmospheric air (ng/m^3)	0.68 ± 0.37	0.15 ± 0.06	<0.0001
1-Nitropyrene in the atmospheric air (pg/m^3)	1.31 ± 0.54	0.44 ± 0.44	<0.0001
TBARS in the urine (μM/g creatinine)	1.51 ± 0.80	2.74 ± 1.39	<0.0001
8-Hydroxydeoxyguanosine in the urine (μg/g creatinine)	1.16 ± 0.70	1.44 ± 0.89	0.2142
1-OHP in the urine (μg/g creatinine)	0.04 ± 0.04	0.04 ± 0.06	0.9259
6-OHNP in the urine (ng/g creatinine)	4.30 ± 6.44	1.65 ± 2.15	0.0269
1-NAAP in the urine (ng/g creatinine)	0.20 ± 0.18	0.35 ± 0.52	0.1753

1-OHP, 1-hydroxypyrene; 6-OHNP, 6-hydroxy-1-nitropyrene; 1-NAAP, N-acetyl-1-aminopyrene.

The results of the correlation analysis of PAHs for TBARS and 8-OHdG are shown in Figure 1 and Figure 2. BaP was significantly associated with the 8-OHdG urinary concentration in summer. The 1-NP atmospheric air concentration and 1-OHP and 6-OHNP urinary concentrations were not significant determinants for urinary TBARS and 8-OHdG concentrations. The 1-NAAP urinary concentration showed a significant positive association with the 8-OHdG urinary concentration in summer.

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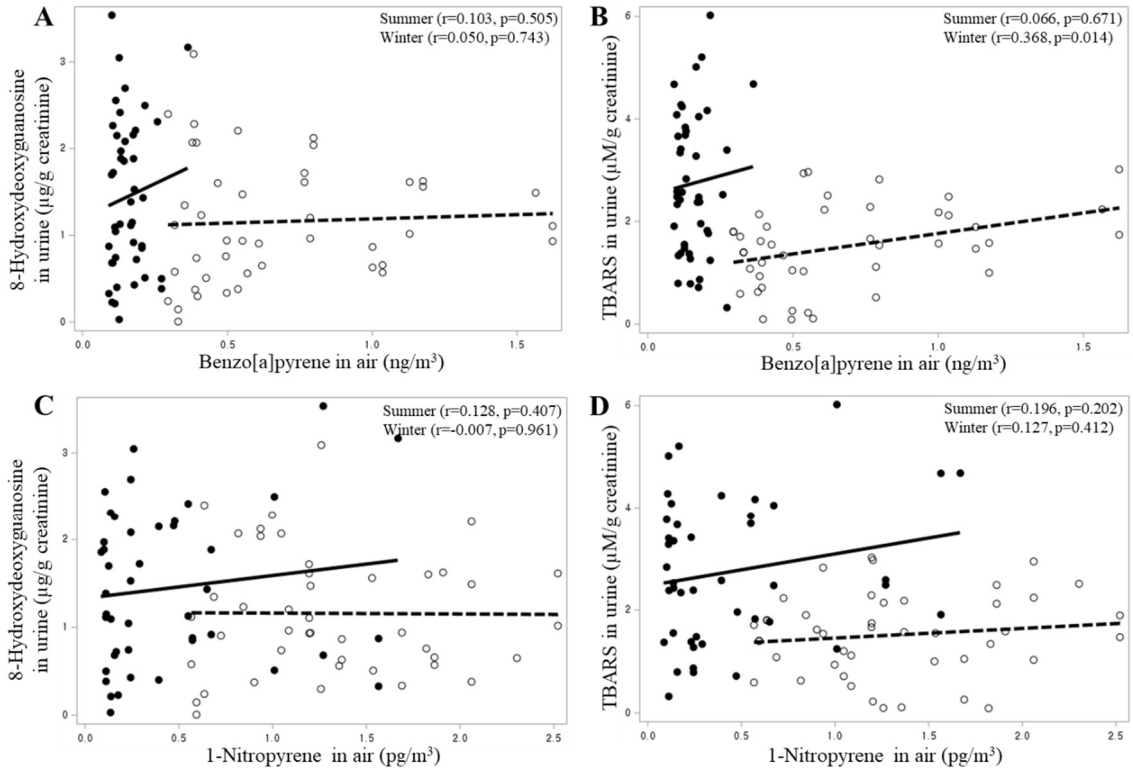

Figure 1. Correlation of atmospheric benzo[a]pyrene (**A, B**) and 1-nitropyrene (**C, D**).concentrations with urinary 8-hydroxydeoxyguanosine and TBARS concentrations (Winter: open circles and dashed lines. Summer: solid circles and solid lines).

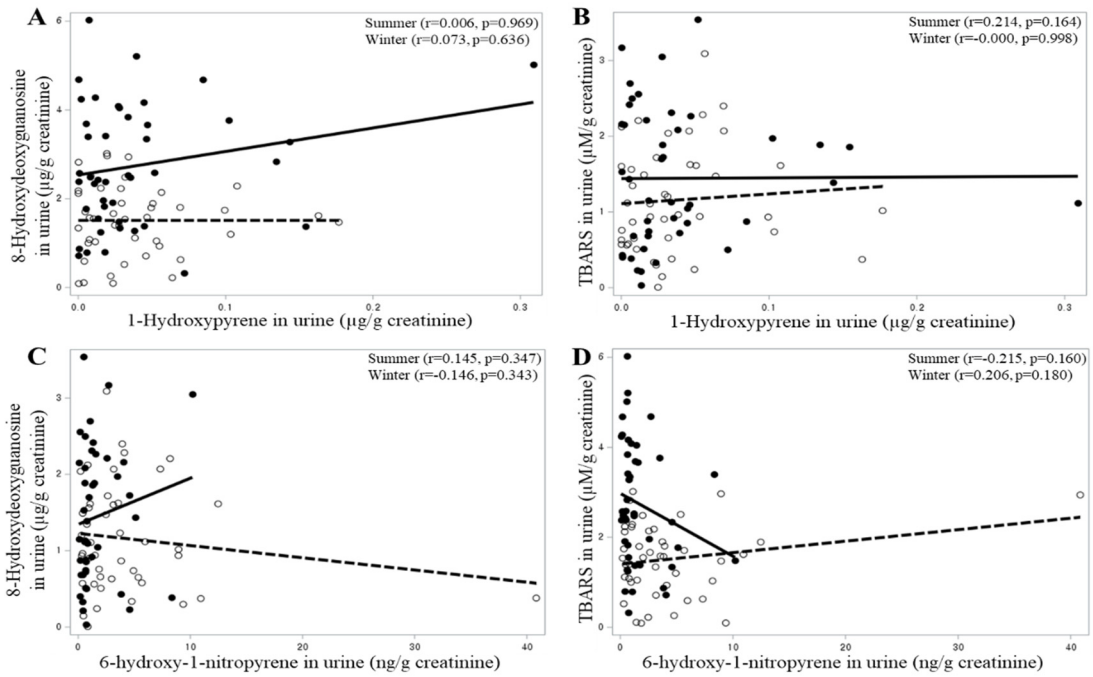

Figure 2. Correlation of urinary 1-hydroxypyrene (**A, B**), 6-hydroxy-1-nitropyrene (**C, D**), N-acetyl-1-aminopyrene (**E, F**) concentrations with 8-hydroxydeoxyguanosine and TBARS concentrations (Winter: open circles and dashed lines. Summer: solid circles and solid lines).

The results of the general linear model analysis are shown in Table 3. Current smoking status was associated with urinary TBARS and 8-OHdG concentrations in summer. The cumulative

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smoking amount was negatively associated with TBARS urinary concentration in summer. In the multivariate model, BaP was significantly associated with the TBARS urinary concentration in winter and the 8-OHdG urinary concentration in summer. The 1-NP atmospheric air concentration and 1-OHP and 6-OHNP urinary concentrations were not significantly associated with TBARS and 8-OHdG urinary concentrations in any season. The 1-NAAP urinary concentration was positively associated with the 8-OHdG urinary concentration in both seasons.

Table 3. General linear model for TBARS and 8-hydroxydeoxyguanosine.

Variables	Season	TBARs in urine (μM/g creatinine)		8-OHdG in urine (μg/g creatinine)	
		B	p-value	β	p-value
Current smoking (no=0, yes=1)	Winter	−0.106	0.7962	−0.454	0.2485
	Summer	14.41	0.0412	0.990	0.0466
Cumulative smoking amount (Pack year)	Winter	0.011	0.2994	0.018	0.0748
	Summer	−0.052	0.0048	0.013	0.3089
Benzo[a]pyrene in air (ng/m^3)	Winter	0.760	0.0251	0.207	0.8833
	Summer	5.566	0.1158	5.496	0.0313
1-Nitropyrene in air (pg/m^3)	Winter	−0.271	0.2531	−0.156	0.5174
	Summer	0.345	0.4593	−0.309	0.3511
1-OHP in urine (μg/g creatinine)	Winter	−432.7	0.1662	276.6	0.3503
	Summer	407.0	0.4167	−657.5	0.0651
6-OHNP in urine (ng/g creatinine)	Winter	0.762	0.7038	−2.803	0.1482
	Summer	−11.22	0.2212	6.256	0.3324
1-NAAP in urine (ng/g creatinine)	Winter	49.08	0.4670	134.2	0.0373
	Summer	39.72	0.2899	55.69	0.0300

1-OHP, 1-hydroxypyrene; 6-OHNP, 6-hydroxy-1-nitropyrene; 1-NAAP, N-acetyl-1-nitropyrene.

4. Discussion

The atmospheric air concentration of BaP or 1-NP was higher in winter than that in summer. This is not only because more fossil fuels are burned for heating in winter but also because considerable amounts of PM generated in foreign countries are brought across the border by the northwest wind. Nevertheless, the participants' TBARS and 8-OHdG urinary concentrations were higher in summer than those in winter, and the TBARS urinary concentration was significantly different. Jacobs et al. (2020) reported that under high temperature environments, high temperature and dehydration increase oxidative damage and induce anti-oxidant defense [10].

BaP exposure was significantly associated with oxidative stress marker concentrations. However, the urinary concentration of 1-OHP, which is widely used as a biomarker for PAH exposure, did not show a significant correlation with TBARS and 8-OHdG urinary concentrations. Since 1-OHP is a pyrene metabolite, which is neither carcinogenic nor mutagenic, the 1-OHP concentration would not show a significant relationship with those oxidative stress markers. We observed no significant correlation between the BaP atmospheric air concentration and 1-OHP urinary concentration of the participants ($r = 0.059$, $p = 0.586$). If the urinary concentration of BPDE, a specific exposure marker for BaP, was measured, a significant association was likely to be observed.

The level of exposure to 1-NP and the urinary concentration of 6-OHNP, which is a 1-NP metabolite, did not show a significant correlation with the urinary concentrations of those oxidative

stress markers. However, the urinary concentration of 1-NAAP, another 1-NP metabolite, was significantly associated with the 8-OHdG urinary concentration. In the human body, absorbed 1-NP is mostly metabolized to 6-OHNP, 8-OHNP, or 3-OHNP, and <5% is metabolized to 1-AP or 1-NAAP. These results suggest that 8-OHdG is produced when 1-NP is metabolized to 1-AP or 1-NAAP but not when 1-NP is metabolized to 6-OHNP. The 1-NP atmospheric air concentration showed a significant association with the 6-OHNP urinary concentration ($r = 0.291$, $p = 0.006$) but not with that of 1-NAAP ($r = -0.067$, $p = 0.534$). Additionally, the relationship between 6-OHNP and 1-NAAP urinary concentrations was not significant ($r = -0.109$, $p = 0.312$). The proportion of 1-NP metabolized to 1-AP or 1-NAAP in the body is determined by the genetic polymorphism of the metabolic enzyme [11]. Therefore, in an individual, if the exposure level to 1-NP increases, the urinary 1-AP or 1-NAAP concentration and, accordingly, the 8-OHdG urinary concentration increase; however, in comparison between individuals, a higher exposure level to 1-NP does not necessarily mean higher 1-AP, 1-NAAP, or 8-OHdG urinary concentrations.

While MDA and 8-OHdG are both biomarkers for oxidative stress, they reflect different aspects of oxidative damage. MDA is produced by lipid peroxidation, and high MDA levels are associated with cardiovascular disease, inflammation, and oxidative stress-related diseases. Since 8-OHdG is produced by ROS attacking DNA, 8-OHdG represents the degree of oxidative stress on DNA. Increased 8-OHdG concentrations in the body indicate increased oxidative damage to DNA, which is associated with various conditions, including aging, cancer, cardiovascular disease, and neurodegenerative disorders [12]. BaP exposure showed a significant positive correlation with not only the 8-OHdG urinary concentration but also the TBARS urinary concentration; however, the 1-NAAP urinary concentration was statistically significant only with the 8-OHdG urinary concentration. These suggest that there may be a slight difference between the adverse health effects that can be caused by BaP exposure and those by 1-NP exposure.

5. Conclusions

Oxidative stress in the body increases in proportion to inhalation exposure to BaP, and more 8-OHdG is produced in the body as the amount of 1-NP, which is metabolized to 1-AP or 1-NAAP, increases.

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