ORIGINAL ARTICLE

Exposure to traffic exhausts and oxidative DNA damage

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Aims: To assess the relations between exposure to traffic exhausts and indicators of oxidative DNA damage among highway toll station workers.**Methods:** Cross-sectional study of 47 female highway toll station workers exposed to traffic exhausts and 27 female office workers as a reference group. Exposure assessment was based on average and cumulative traffic density and a biomarker of exposure, urinary 1-hydroxypyrene-glucuronide (1-OHPG). Urinary 8-hydroxydeoxyguanosine (8-OHdG) was used as a biomarker of oxidative DNA damage. Plasma nitric oxide (NO) was measured as an indicator of oxidative stress related to traffic exhaust exposure.**Results:** The mean concentration of urinary 8-OHdG was substantially higher among the exposed non-smokers (13.6 µg/g creatinine) compared with the reference non-smokers (7.3 µg/g creatinine; difference 6.3, 95% CI 3.0 to 9.6). The mean concentration of NO among the exposed (48.0 µmol/l) was also higher compared with the reference non-smokers (37.6 µmol/l; difference 10.4, 95% CI –0.4 to 21.2). In linear regression adjusting for confounding, a change in log(8-OHdG) was statistically significantly related to a unit change in log(1-OHPG) ($\beta=0.372$, 95% CI 0.081 to 0.663).**Conclusions:** Results indicate that exposure to traffic exhausts increases oxidative DNA damage. Urinary 8-OHdG is a promising biomarker of traffic exhaust induced oxidative stress.

Traffic exhausts constitute a complex mixture of by-products of the combustion process including hundreds of pollutants in gaseous and particulate phases. Most of the particulate phase constitutes fine-mode particulate matter, which is derived from combustion processes that has volatilised and then condensed to form primary particles or from precursor gases reacting in the atmosphere to form secondary particles. Epidemiological studies have suggested that exposure to atmospheric particulate matter is associated with an increased risk of morbidity and mortality, but the mechanism remains unclear.^{1–2}

It has been suggested that reactive oxygen species (ROS) and reactive nitrogen species (RNS) are involved in aging, mutagenesis, and carcinogenesis.^{3–4} ROS include superoxide, hydrogen peroxide, and hydroxyl radical. RNS include nitric oxide and its derivatives such as nitrogen dioxide and peroxyxynitrite. Particles in traffic exhausts can produce a significant amount of ROS. The smaller particles in ambient aerosols have higher ROS contents.⁵ Fine particles from traffic exhausts also contain polycyclic aromatic hydrocarbons (PAHs),^{6–8} and some of them are known or suspected human carcinogens.⁹ Pyrene is commonly found in mixtures of PAHs; it has metabolites such as 1-hydroxypyrene (1-OHP) and 1-hydroxypyrene-glucuronide (1-OHPG), which can be measured in human urine. These metabolites have previously been used as biomarkers of recent exposure to traffic exhaust; recent findings indicate that exposure to traffic exhausts increases the urinary concentration of 1-OHPG with a dose-response pattern suggesting that it might be a useful biomarker for vehicle exhaust exposure.¹⁰ The intermediate metabolites of PAH having electrophilic properties can covalently attach to intracellular macromolecules, leading to the generation of ROS, and thus possibly contributing to the development of disease.^{11–12} Nitric oxide (NO) is a free radical gas that plays an important role in neurotransmission, vasodilatation, and immune defence.¹³ It is synthesised from L-arginine by nitric oxide synthase. There is evidence that oxidative stress induced by traffic exhaust is associated with the impairment of NO production.^{12–14} Plasma

nitrite levels, representative of NO production, are determined in plasma. In pulmonary vessels, NO is a vasodilator, but in excess it may be cytotoxic and cause further damage to the surrounding epithelium and endothelium.^{13–14} In airways epithelium, NO modulates the immune response.

Attacks on DNA by reactive oxygen species frequently result in oxidative DNA damage. Urinary 8-hydroxydeoxyguanosine (8-OHdG) is a biomarker of oxidative DNA stress, reflecting its repair from DNA. 8-OHdG is a modified base that occurs in DNA due to attack by hydroxyl radicals that are formed as products and intermediates of aerobic metabolism and during oxidative stress. Biomonitoring in humans has shown that 8-OHdG may correlate with oxidative stress and damage to DNA.^{15–17}

The overall objective of the present study was to study the relation between exposure to high levels of traffic exhausts and the occurrence of oxidative DNA damage. We assessed the exposure using measures of traffic density and urinary 1-OHPG. Urinary 8-OHdG was used as a measure of oxidative DNA damage. We also assessed the levels of plasma NO as a biomarker of oxidative stress related to traffic exhaust exposure.

MATERIALS AND METHODS

Study population

We carried out a cross-sectional study among female toll station workers with occupational exposure to traffic exhausts and a reference group of female workers who were in classroom training to become toll station workers, which has been described previously.¹⁰ Briefly, we recruited all toll station workers in a highway toll station in the Taipei metropolitan area, Taiwan. The toll station is located on the First Highway, 10 km south from Taipei City. According to

Abbreviations: 1-OHP, 1-hydroxypyrene; 1-OHPG, 1-hydroxypyrene-glucuronide; 8-OHdG, 8-hydroxydeoxyguanosine; eNOS, endothelial nitric oxide synthase; iNOS, inducible nitric oxide synthase; NO, nitric oxide; PAHs, polycyclic aromatic hydrocarbons; ROS, reactive oxygen species; RNS, reactive nitrogen species

Main messages

- Subjects exposed to high levels of traffic exhausts had substantially higher levels of urinary 8-OHdG and plasma NO compared with the reference group.
- Traffic exhausts, possibly metabolites of PAHs, may form an electrophilic derivative that binds to DNA and may involve ROS. Such changes may include ROS induced DNA strand breaks, base modifications, oxidative modification of DNA, and result in increasing urinary 8-OHdG levels.
- Exposure to traffic exhausts may induce endothelial cell nitric oxide synthase, leading to increase in plasma NO concentrations and pathophysiological changes in vascular tone.

the Bureau of Highways records, this toll station had the highest traffic density among all toll stations in Taiwan. All the toll workers work in three shifts: morning (from 8 00 am to 4 00 pm), evening (from 4 00 pm to 00 00 am), and night shift (from 00 00 am to 8 00 am). There are 20 tollbooths—10 are used to collect the toll of traffic flow from Taipei City to Taipei County (from north to south), and the remaining 10 booths collect toll from the opposite traffic flow. Of the 10 booths in both directions, 2–3 were designed for bus and truck traffic. The other 7–8 booths were for cars and vans. These lanes are divided into those using prepaid tickets and those using cash payment. The number of open booths depends on the traffic flow. The workers followed a schedule, which defined the shift and the type of lane. As a rule, they worked four days in a row and then took one day off. There was a continuous rotation of lanes from one day to another. The duration of the working day was eight hours and the workers were allowed to take a 30–45 minute break every 1–2 hours. The exposed group included 47 toll station workers. The reference group consisted of 27 office workers.

Exposure assessment

Exposure assessment was based on measures of traffic density and urinary biomarker levels. Traffic density was expressed as both dichotomous and continuous variables. The dichotomous exposure variable was based on the type of work: toll collection or office work. The continuous exposure variables were based on source strength in the microenvironment and time-activity of the worker during the working day. First, we applied average lane specific traffic density in vehicles per hour, separating the vehicles into buses and trucks or personal cars. Second, we assessed cumulative exposure by multiplying traffic density (vehicles per hour) by the time spent at the toll booth (hours); the unit was expressed in the number of vehicles per working day. The biomarker of exposure to traffic exhaust was urinary concentration of 1-hydroxypyrene-glucuronide (1-OHPG) after the working shift.

Outcome assessment

The outcome of interest was oxidative DNA damage. We used the concentration of 8-hydroxydeoxyguanosine (8-OHdG) in the urine after the working shift as the biomarker of effect of exposure to traffic exhausts on DNA. In addition, we used plasma nitric oxide (NO) after the working shift as a biomarker of effect on NO production.

Data collection

In the beginning of the study, we distributed a self-administered questionnaire to the participants, which

Policy implications

- Current levels of occupational exposure to traffic exhausts from motor vehicles should be reduced.

inquired about personal characteristics, such as age, education, marital status, and smoking habits; mode of transportation to work; a history of diseases; consumption of broiled, grilled, and barbecued food; use of cooking fuel; cooking practice; use of incense, candles, and mosquito coils; and use of personal protective equipment. Workers were asked to collect a post-shift urine sample in a container. Venous blood was also collected post-shift, drawn into Vacutainers (Becton Dickinson, Rutherford, New Jersey) containing sodium heparin, centrifuged at 4°C for 10 minutes at 1200 *g*, protected from light, and stored at –80°C.

This study was approved by the institutional review boards at the Tri-Service General Hospital, Taipei, Taiwan. All the participants signed informed consent prior to study enrolment.

Analysis of urinary 1-OHPG

The urine samples were collected in brown polyethylene 500 ml containers and labelled with subject identification number, date, and time. The samples were transported in a cooler. Urine samples were divided into several small volume aliquots and stored at –80°C to minimise the effect of freeze-thaw on the stability of specimens. Urine samples (2 ml) were treated with 0.1 N HCl (90°C) to hydrolyse acid labile metabolites, as described previously.¹⁸ The hydrolysed samples were loaded onto Sep-Pak C18 cartridges (Waters) and washed with methanol (30% in water). The relatively non-polar metabolites were eluted with methanol (80% in water; 4 ml) and the volume was reduced to 0.5 ml by a centrifugal and vacuum evaporator (Eyela CVE-100, Tokyo, Japan). The concentrated samples were diluted to 4 ml with 15 mM phosphate buffered saline (PBS). Immunoaffinity columns were prepared using poly-prep columns (0.8×4 cm) filled with CNBr activated Sepharose 4B (0.8 ml) coupled with monoclonal antibody 8E11, which recognises several PAH-DNA adducts and metabolites. Monoclonal antibody 8E11 was obtained from Trevigen, Inc., Gaithersburg, MD, USA. It was originally produced against benzo[a]pyrene-diolepoxide modified DNA, and has been shown to recognise 1-OHPG.¹⁸ After washing the columns twice with 4 ml of 15 mM PBS, samples in phosphate buffered saline were loaded on columns and bound material was eluted with 2 ml of 40% methanol. Eluted fractions were quantified by synchronous fluorescence spectroscopy (SFS) using a Perkin-Elmer LS 50B luminescence spectrometer. The excitation-emission monochromators were driven synchronously with a wavelength difference of 34 nm. 1-OHPG, purchased from the National Cancer Institute (NCI) Chemical Carcinogen Repository (MRI, Kansas City, MO, USA), produces a characteristic fluorescence emission maximum at 381 nm (347 nm excitation). Fluorescence intensity was used to quantify 1-OHPG, as described previously.^{18,19} The recovery of the assay was 80%. The coefficient of variation of the assay was 8–10% during the period of sample analysis. The limit of detection was 0.06 pmol/ml as determined by the concentration of the standard at which the signal-to-noise ratio was 3. The urinary 1-OHPG concentrations were normalised to urine creatinine. Creatinine was determined spectrophotometrically (Perkin-Elmer Lambda 5 model) with a commercial kit (Boehringer, Mannheim, Germany) based on Jaff's basic picrate method.

Analysis of 8-OHdG

Levels of urinary 8-OHdG were determined by indirect competitive enzyme linked immunosorbent assay (ELISA) (New 8-OHdG Check; Institute for the Control of Aging, Fukuroi, Shizuoka, Japan) according to Chuang and colleagues¹⁹ and Maeshima and colleagues.²⁰ Urine samples were thawed and centrifuged at 2000 rpm for 10 minutes to obtain the opaque layer. A 50 µl aliquot of sample or standard and 50 µl of primary monoclonal antibodies were added to microtitre plates pre-coated with 8-OHdG. The plates were then incubated at 37°C for 1 hour with continuous mixing at 100 rpm. The bounding of free 8-OHdG were washed with 250 µl phosphate buffered saline. Horseradish peroxidase (HRP) conjugated secondary antibodies (100 µl) were added to each well and then the plates were incubated for 1 hour with mixing. The unbound secondary antibody was washed away. A 100 µl aliquot of chromatic substrate was added to each well and incubated in the dark at room temperature for 15 minutes; 100 µl of 1.0 M phosphoric acid was added to terminate the reaction. The absorbance was measured at 450 nm with a computer controlled ELISA reader (MRXII, Dynex Technologies, VA, USA). The concentration of 8-OHdG of the urine samples was interpolated from a standard curve drawn with the assistance of logarithmic transformation. The coefficient of variation of the assay was within 6% during the period of sample analysis. The results were expressed as µg/g creatinine.

Analysis of plasma nitric oxide

To one aliquot of plasma 100 µl ethanol was added; it was then centrifuged for 30 minutes at 15 000 *g*. To avoid potential contamination due to storage, a volume of 10 µl of supernatant was immediately assayed by the NO/ozone chemiluminescence technique (NO-Analyzer 280 A, Sievers Research Inc., Boulder, CO) as described previously.²¹ Briefly, we measured the oxidation products (NO_2^-) of NO using a reaction vessel containing a reducing system (0.1 M vanadium chloride, Aldrich Co., Germany). Detection of NO was then completed by its reaction with ozone, which lead to the emission of red light ($\text{NO} + \text{O}_3 \rightarrow \text{NO}_2^* + \text{O}_2$; $\text{NO}_2^* \rightarrow \text{NO}_2 + \text{hv}$). Standard curves were made before each measurement by a mean of three injections of each concentration (1, 3, 5, 10 µM NO), which were prepared using freshly prepared solutions of NaNO_2 (10 µl) in distilled water. The correlation between the above mentioned four nitrite concentrations and the integrated areas of their peaks was linear. The correlation coefficient was 0.984. The results were expressed as µmol/l.

Statistical methods

First, we compared the distributions of 8-OHdG and NO concentrations between the exposed and the reference group. The 8-OHdG concentrations were roughly normally distributed (skewness 1.07, kurtosis 4.20, Shapiro-Wilk W test for normal data $p=0.09$) and therefore we assessed the difference in means using the unpaired *t* test. We also compared the distributions using a non-parametric Wilcoxon rank-sum test. We estimated the relation between the 8-OHdG concentration and the determinants of interest in linear regression analysis adjusting for confounding. Second, we studied the relations of traffic density to urinary 8-OHdG and plasma NO concentrations in linear regression models. We fitted the two types of exposure variables described above: average traffic density (vehicles per hour) and cumulative traffic density (number of vehicles). The covariates used in the analyses included: age (18–24, 25–29, 30–), marital status (married versus unmarried), education (college versus high school), use of motorcycle as main transportation (yes/no), frequent exposure to incense (yes/no), and frequent use of a protective mask (yes/no). We also

fitted similar models using log transformed outcome variables. Finally, we elaborated the relation of 8-OHdG to 1-OHPG, and NO using plots and linear regression analysis adjusting for potential confounders such as age, marital status, smoking, use of motorcycle, and burning of incense. The regression diagnostics indicated heteroscedasticity—that is, increasing variance of the dependent variable by increasing magnitude of the independent parameters (for both relations). We applied log transformations to meet the assumptions of linear regression, which improved the fit in both cases. All the analyses were performed with the STATA 7 statistical package.

RESULTS

Study population

Characteristics of the study population were compared between the exposed and the reference workers (table 1). The mean age was 25.7 years (SD 5.71) in the exposed and 27.0 years (SD 4.71) in the reference workers. The exposed subjects were less often married (6% *v* 30%) and had a higher educational background than the reference group. The prevalence of current smoking was lower among the exposed (11%) compared with the reference group (30%). Among current smokers, the mean daily smoking rate was lower among the exposed (8 cigarettes/day) than among the reference group (13 cigarettes/day). The exposed subjects used a motorcycle less often for transportation and were less frequently exposed to incense burning compared with the reference group. The mean BMI was 21.2 kg/m² (SD 3.2) in the exposed and 21.3 kg/m² (SD 4.1) in the reference group.

Comparison of urinary 8-hydroxydeoxyguanosine and plasma nitric oxide between exposed and reference groups

The percentage of smokers was greater in the reference group (30%, $n=8$) than in the exposed group (11%, $n=5$). To exclude the smoking effect, a stratified analysis was performed. The mean concentration of urinary 8-OHdG was 10.5 µg/g creatinine (SD 8.2) among the exposed smokers and 10.2 µg/g creatinine (SD 4.6) in the reference smokers. The mean concentration of urinary 8-OHdG was 13.6 µg/g creatinine (SD 7.3, median 15.1, 25th centile 8.2, 75th centile 18.3) among the exposed subjects and 7.3 µg/g creatinine (SD 4.8, median 7.7, 25th centile 3.2, 75th centile 10.3) in the reference group. The difference, 6.3 µg/g creatinine (95% CI 2.98 to 9.64), was not likely to be explained by chance (*t* test: $p=0.004$; Wilcoxon rank sum test: $p=0.0022$). We fitted a linear regression model for 8-OHdG adjusting for the covariates in table 1. The adjusted difference in the mean urinary 8-OHdG concentration was 4.68 (95% CI 0.14 to 9.21).

The mean concentration of plasma nitric oxide was 58.7 µmol/l (SD 19.5) among the exposed smokers and 40.1 µmol/l (SD 10.0) in the reference smokers. The mean concentration of NO was 48.0 µmol/l (SD 23.8, median 42.6, 25th centile 28.4, 75th centile 64.1) among the exposed non-smokers and 37.58 µmol/l (SD 14.1, median 37.4, 25th centile 27.4, 75th centile 47.4) in the reference non-smokers with a difference of 10.48 µmol/l (95% CI –0.4 to 21.2) (unpaired *t* test: $p=0.11$; Wilcoxon rank sum test: $p=0.25$).

When NO data from smokers and non-smokers were analysed together, the mean concentration for the exposed workers was 49.1 (SD 23.4 µmol/l) and for the unexposed workers 38.4 (SD 12.7 µmol/l) (*t* test: $p=0.03$; Wilcoxon rank sum test: $p=0.16$). When we fitted a linear regression model for NO adjusting for the covariates presented in table 1 the adjusted difference in the mean NO concentration was 18.78 (95% CI 5.48 to 32.08).

Table 1 Characteristics of the study population

Characteristic	Exposed		Reference		Total		p value
	n	%	n	%	n	%	
Number of subjects	47	63.5	27	36.5	74	100	
Age							
18–24	24	51.1	7	25.9	31	41.9	0.04
25–29	10	21.3	13	48.2	23	31.1	
30–	13	27.6	7	25.9	20	27.0	
Marital status							
Married	3	6.4	8	29.6	11	14.9	0.007
Single	44	93.6	19	70.3	63	85.1	
Education							
High school	30	63.8	26	96.3	56	75.7	0.002
College	17	36.2	1	3.7	18	24.3	
Current smoking habits							
Smokers	5	10.6	8	29.6	13	17.6	0.03
Non-smokers	42	89.4	19	70.4	61	82.4	
Transportation							
Motorcyclist	6	12.8	16	59.3	22	29.7	<0.001
Others	41	87.2	11	40.7	52	70.3	
Daily use of incense							
Yes	8	17.0	16	59.3	24	32.4	<0.001
No	39	83.0	11	40.7	50	67.6	
BMI (kg/m ²)*	21.23 (3.22)		21.32 (4.07)		21.26 (3.52)		0.47

*BMI, body mass index. Values shown are mean (SD).
Comparison between the exposed and reference groups.

Relations between average traffic density and urinary 8-OHdG and plasma nitric oxide concentrations

We examined the relation between traffic density and the urinary concentration of 8-OHdG. We estimated the relations between the average and the cumulative exposure to traffic exhausts and the concentration of 8-OHdG in linear regression adjusting for potential confounding factors (table 3). The estimates were given for non-smokers (n = 42) and separately for buses and trucks (n = 13) and personal cars (n = 17). The 8-OHdG concentration was related to exposure to both personal cars and trucks and buses, although neither was statistically significant (table 3). There was a 9.32 µg/g creatinine (95% CI –11.59 to 30.23) increase per 1000 cars/hour increase in average traffic density. The corresponding estimate for trucks and buses was of greater magnitude, 16.76 (95% CI –13.63 to 47.16). There was an increase of 8-OHdG in relation to cumulative exposure to trucks and buses (7.10 µg/g creatinine per 1000

vehicles, 95% CI –7.16 to 21.35), but no apparent association for personal cars (0.67, 95% CI –2.45 to 3.79). There was a 0.38 µg/g creatinine increase per 1000 cars/hour (95% CI –0.55 to 1.30) increase in average traffic density. The corresponding estimate for trucks and buses was of lesser magnitude, 0.15 µg/g creatinine (95% CI –1.30 to 1.59). Table 3 presents the similar models using a log transformed outcome variable. There was a non-significant increase of log(8-OHdG) in relation to cumulative exposure to trucks and buses (0.13 µg/g creatinine per 1000 vehicles, 95% CI –0.50 to 0.76), but no apparent association for personal cars (0.07, 95% CI –0.07 to 0.22).

The plasma NO concentration was related to both personal cars and trucks and buses, although neither was statistically significant (table 4). There was a 2.65 µmol/l (95% CI –92.82 to 98.12) increase per 1000 cars/hour increase in average traffic density. The corresponding estimate for trucks and buses was of greater magnitude (160.48, 95% CI –19.56 to

Table 2 Concentration of plasma NO, and urinary 1-OHPG and 8-OHdG measured for exposed and reference groups by smoking status

	Exposed group		Reference group		p value*
	n	Mean (SD)	n	Mean (SD)	
8-OHdG					
Smokers	5	10.2 (4.6)	8	10.5 (8.2)	0.94
Non-smokers	42	13.6 (7.3)	16	7.3 (4.8)	0.0004
Subtotal	47	13.3 (7.1)	24	8.4 (6.2)	0.004
p value†		0.19		0.34	
NO					
Smokers	4	58.7 (19.5)	8	40.1 (10.0)	0.15
Non-smokers	35	48.0 (23.8)	16	37.6 (14.1)	0.06
Subtotal	39	49.1 (23.4)	24	38.4 (12.7)	0.03
p value†		0.365		0.63	
1-OHPG					
Smokers	5	0.16 (0.10)	8	0.18 (0.07)	0.69
Non-smokers	42	0.12 (0.07)	16	0.07 (0.04)	0.01
Subtotal	47	0.12 (0.08)	24	0.11 (0.07)	0.63
p value†		0.35		0.003	

Values shown are mean (SD).

*Comparison between the exposed and reference groups.

†Comparison between the smokers and non-smokers.

8-OHdG, 8-hydroxy-2'-deoxyguanosine (µg/g creatinine); NO, nitrogen oxides (µmol/l); 1-OHPG, 1-hydroxypyrene-glucuronide (µmol/mol creatinine).

Table 3 Relations between traffic density and the urinary 8-OHdG ($\mu\text{g/g}$ creatinine) concentration in the toll station workers among non-smokers ($n = 42$)

	Increase in 8-OHdG				Increase in log(8-OHdG)			
	Unadjusted		Adjusted*		Unadjusted		Adjusted*	
	B	95% CI	B	95% CI	B	95% CI	B	95% CI
Average exposure to cars (per 1000 vehicles/hour)	4.16	-15.64 to 23.96	9.32	-11.59 to 30.23	0.20	-1.05 to 1.45	0.38	-0.55 to 1.30
Average exposure to trucks and buses (per 1000 vehicles/hour)	19.15	-8.11 to 46.41	16.76	-13.63 to 47.16	0.58	-1.03 to 2.19	0.15	-1.30 to 1.59
Cumulative exposure to cars (per 1000 vehicles)	0.15	-2.62 to 2.92	0.67	-2.45 to 3.79	0.03	-0.15 to 0.20	0.07	-0.07 to 0.22
Cumulative exposure to trucks and buses (per 1000 vehicles)	-0.09	-0.82 to 0.64	7.10	-7.16 to 21.35	-0.09	-0.824 to 0.64	0.13	-0.50 to 0.76

*Adjusted for age, use of motorcycle, daily burning of incense, and wearing of mask.

340.53). The adjusted increase of NO in relation to cumulative exposure per 1000 vehicles was also greater for trucks and buses (29.11, 95% CI -17.98 to 76.20) compared to personal cars (4.97, 95% CI -9.40 to 19.34). A similar pattern was found when using logNO as the outcome variable, as shown in table 4.

Relations of urinary 8-OHdG to urinary 1-OHPG and plasma NO

A plot presentation indicated a monotonous increase of 8-OHdG by 1-OHPG. The graphical presentation as well as regression diagnostics indicated strong heteroscedasticity, which was corrected by log-log transformation. There was a significant linear relation between log(8-OHdG) and log(1-OHPG), as shown in table 5. In linear regression adjusting for confounding, a change in log(8-OHdG) was statistically significantly related to a unit change in log(1-OHPG) ($\beta = 0.372$, 95% CI 0.081 to 0.663). Adjustment for confounding weakened the relation only slightly. There was no significant association between 8-OHdG and NO or corresponding log transformed parameters.

DISCUSSION

The findings of the present study indicate an increased amount of DNA damage measured using urinary 8-OHdG in subjects working under conditions of potential oxidative stress due to exposure to traffic exhausts. Non-smoking female toll workers exposed to traffic pollutants had on average 90% and 30% higher levels of urinary 8-OHdG and plasma NO, respectively, compared with female office workers. There was a statistically significant linear relation between the log transformed urinary exposure biomarker (1-OHPG) and the urinary measure of DNA damage (8-OHdG). This relation did not change substantially when

adjusting for determinants of 8-OHdG such as cigarette smoking and use of motorcycle in transportation.

Validity of results

In order to achieve comparability of the two groups, we selected a reference group from women who were in training to become toll station workers, but not yet exposed to high levels of traffic exhausts at work. Nevertheless, the two occupational groups differed in certain potential determinants of urinary 8-OHdG, which might have influenced the comparison. The groups were comparable as to gender (all women) and age. However, smoking was a strong determinant of urinary 8-OHdG levels and there were more smokers among the office workers (Pearson's $\chi^2 = 4.27$, $p = 0.039$) chosen for the reference group compared with toll workers considered to be exposed. Restricting the analyses to non-smokers eliminated the influence of smoking. We also applied linear regression analysis to adjust for other potential confounders. In assessing the relation between traffic density and 8-OHdG and NO levels among the exposed toll station workers, the study design minimised potential selection bias. The traffic lane for the studied workplace was selected for each participant from a rotating schedule. Thus the occupational exposure level was allocated independently from personal characteristics or other determinants of 8-OHdG and NO. The occupational exposure during the studied working days could have been, by chance, related to other factors influencing urinary 8-OHdG and NO; therefore we also fitted multivariate linear regression models with all the potential confounders. In general, the adjustment for covariates strengthened the relation for both truck and bus and car traffic density and the urinary 8-OHdG concentrations. The adjustment had little effect on the relation between personal car traffic density and NO concentrations,

Table 4 Relations between traffic density and the NO ($\mu\text{mol/l}$) concentration in the toll station workers among non-smokers ($n = 42$)

	Increase in NO				Increase in log(NO)			
	Unadjusted		Adjusted*		Unadjusted		Adjusted*	
	B	95% CI	B	95% CI	B	95% CI	B	95% CI
Average exposure to cars (per 1000 vehicles/hour)	16.36	-63.62 to 96.34	2.65	-92.82 to 98.12	0.25	-0.29 to 0.79	0.22	-0.65 to 1.09
Average exposure to trucks and buses (per 1000 vehicles/hour)	127.59	-17.34 to 272.52	160.48	-19.56 to 340.53	0.62	-0.90 to 2.13	1.24	-0.26 to 2.75
Cumulative exposure to cars (per 1000 vehicles)	8.77	-1.09 to 18.63	4.97	-9.40 to 19.34	0.08	0.01 to 0.14	0.08	-0.03 to 0.19
Cumulative exposure to trucks and buses (per 1000 vehicles)	21.19	-13.78 to 56.17	29.11	-17.98 to 76.20	0.15	-0.12 to 0.42	0.26	-0.02 to 0.54

*Adjusted for age, use of motorcycle, daily burning of incense, and wearing of mask.

Table 5 Simple linear regression of log (8-OHdG) by log(1-OHPG) and a general linear regression with potential confounders as covariates (n = 71)

Variable	β	(SE β)*	t	95% CI
Unadjusted relation				
Log(1-OHPG)	0.377	0.121	3.12	0.136 to 0.619
Adjusted relation				
Log(1-OHPG)	0.372	0.145	2.57	0.081 to 0.663
Age	-0.01	0.009	-1.14	-0.028 to 0.008
Marital status (married/single)	-0.274	0.147	-1.86	-0.571 to -0.021
Exposed v reference group	0.129	0.108	1.20	-0.087 to 0.346
Current smoking (smoker/non-smoker)	0.021	0.124	0.17	-0.227 to 0.269
Transportation (motorcyclist/others)	-0.241	0.110	-2.20	-0.461 to -0.022
Daily burning of incense (yes/no)	0.032	0.067	0.48	-0.102 to 0.166

SE β = standard error of β coefficient.

but strengthened the relation for truck and bus traffic. The number of toll station workers was relatively small and non-occupational factors may have had a substantial impact on the urinary 8-OHdG and plasma NO concentrations. Therefore the estimated relations have wide confidence limits.

Synthesis with previous knowledge

We identified several previous studies that have applied urinary 8-OHdG as biomarkers of oxidative stress in general. The formation of 8-OHdG is related to personal physiological factors (age, gender), environmental exposure (traffic exhaust, sunlight), lifestyle (smoking, betel nut chewing), disease (cancer, systemic lupus erythematosus, arthritis, and diabetes mellitus).^{15 16 20 22} Chuang and colleagues¹⁵ compared the levels of urinary 8-OHdG between male taxi drivers and male community residents in Taipei, Taiwan. The average level of urinary 8-OHdG was significantly higher in taxi drivers than in community residents (13.4 (4.7) v 11.5 (4.7) $\mu\text{g/g}$ creatinine, mean (SD)). The average 8-OHdG levels also were higher in smokers than in non-smokers among both the taxi drivers and the community men. In addition, the community men had higher levels of 8-OHdG than that of our reference female group. The 8-OHdG levels for taxi drivers were of similar magnitude compared with our female toll workers. There were no PM_{10} or $\text{PM}_{2.5}$ levels determined in the study. Based on our previous measurements in the Taipei toll station,²³ the workers were exposed to very high levels of particles: the median eight hour concentrations of $\text{PM}_{2.5}$ were from 215 to 346 $\mu\text{g}/\text{m}^3$ in the bus and truck lanes, and from 87 to 130 $\mu\text{g}/\text{m}^3$ in the car lanes, while the control group was exposed to 50–70 $\mu\text{g}/\text{m}^3$.

Squadrito and colleagues²⁴ hypothesise that semiquinone radical, present in $\text{PM}_{2.5}$, will undergo redox cycling, thereby reducing oxygen and generating ROS. These ROS will cause oxidative stress at the site of deposition and produce deleterious effects in the lung. Ulrich and colleagues¹⁴ proposed a possible mechanism for PM induced health effects. Briefly, PM will activate alveolar macrophages and epithelial cells to synthesise cytokines. As a consequence, inflammatory cells will be recruited to enhance the inflammatory process. Meanwhile, inducible NO synthase (iNOS) will be induced in the epithelial cells and endothelium. Phagocytosis of the PM by macrophages results in the release of ROS. The induction of iNOS will release a large amount of NO. Thus, both NO and ROS could cause further damage to the surrounding epithelium and endothelium.

The results of the present and one previous¹⁵ study indicate that plasma NO reflects exposure to traffic related and other types of air pollutants. The average level of plasma NO was higher in the non-smoking drivers (42.4 (6.0) μM) than in the non-smoking community men (37.7 (7.5) μM).¹⁵ In

addition, the NO level among the exposed was 13% higher than that of non-smoking community men, while in our study the difference was 30% higher. The above evidence suggests the increased plasma NO might be induced by endothelial cell nitric oxide synthase (eNOS). Conversely, others suggest that nitric oxide formed by endothelial NO synthase may be suppressed by traffic exhaust particles.¹²

The plasma NO was not associated with urinary 8-OHdG and 1-OHPG. This may be explained by the different kinetics of these parameters. The levels of 8-OHdG are more likely PAH dependent, whereas NO are probably not. Urinary 1-OHPG has been extensively used as a means for assessing recent exposure to PAHs. The half life is about 6–35 hours. The 8-OHdG represents the DNA repair process for damage from exposure over a longer time period. The oxidation of PAHs can form electrophilic derivatives that can attack and bind to DNA and may involve ROS. Such changes may include ROS induced DNA strand breaks, base modifications, oxidative modification of DNA, etc.^{3 24 25} Lai and colleagues⁸ indicated that pyrene concentrations are highest in fine particles. The fine particles in the exhausts of trucks and buses have a higher content of pyrene compared with corresponding size fraction of particles from passenger cars. Trucks and buses constitute a stronger source of pyrene exposure by both producing higher concentrations of fine and coarse particles, and by producing fine particles with higher concentration of pyrene. These might explain why the adjusted increase of 8-OHdG in relation to cumulative or average traffic density was greater for trucks and buses compared to personal cars.

The presence of the 8-OHdG in urine represents the primary repair product of oxidative DNA damage in vivo, presumably nucleotide excision repair.^{26 27} Hu and colleagues²⁸ indicate that N45 overestimates 8-OHdG levels. It is important to note that they also showed that levels determined by ELISA using N45 correlates well with their LC/MS/MS method. Thus, while ELISA is less accurate than the new method, we still see a difference between exposed and non-exposed subjects. It would be of interest to use more advanced methods of analysis for 8-OHdG in these subjects in the future.

There is substantial individual variability in the repair of DNA, which has not been carefully quantified.^{29 30} A comparison of variation within and between individuals in controlled exposure conditions would provide important insight to the usefulness of 8-OHdG as a biomarker of oxidative stress caused by exposure traffic exhausts. Furthermore, this approach does not identify or chemically characterise specific adducts. There is empirical evidence, including genetic and nutritional factors, suggested to influence 8-OHdG metabolism, that was not taken into account in this study. Kristenson and colleagues¹⁶ reported

that lower serum antioxidants in Lithuanian men, such as α - and β -carotene, were inversely correlated to urinary 8-OHdG. The human MutT homologue (hMTH1) is a representative of the class of 8-OHdG repair enzymes, which prevents the cell from incorporating 8-OH-Gua into DNA by hydrolysing 8-OH-dGTP to 8-OH-dGMP.³¹

Conclusions

Our results indicate that exposure to traffic exhausts increases the urinary concentration of 8-OHdG, suggesting that it might be a useful biomarker of oxidative stress from vehicle exhaust exposure. Exposure to traffic exhausts may induce endothelial cell nitric oxide synthase, leading to increase in plasma NO concentrations and pathophysiological changes in vascular tone. Further studies are needed to elaborate the within individual relation between exposure to traffic exhausts and urinary 8-OHdG concentration and to clarify the source of plasma NO.

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