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Ultrafine particulate matter and high-level benzene urban air pollution in relation to oxidative DNA damage

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Air pollution, containing high-level of ultrafine particles (UFP) and benzene, is a prominent environmental health problem in many cities of the World. We investigated the level of oxidative DNA damage in mononuclear blood cells (MNBC) by the comet assay as DNA strand breaks (SB) and formamidopyrimidine DNA glycosylase (FPG) sensitive sites in residents from three urban locations in Cotonou, Benin (taxi-moto drivers, subjects living near roads with intense traffic and suburban residents) and rural residents. Exposure was characterized by urinary excretion of *S*-phenylmercapturic acid (S-PMA), a biomarker of benzene exposure, and by ambient UFP. There were clear stepwise gradients with respect to ambient UFP, S-PMA excretion and oxidative DNA damage with rural subjects < suburban subjects < residents living near highly trafficed roads < taxi-moto drivers. Polymorphisms in glutathione peroxidase (GPX), NAD(P)H:quinone oxidoreductase 1 (NQO1) and glutathione *S*-transferase (GST) genes were assessed for effect modification. Subjects with GSTT1 null genotype had lower urinary S-PMA excretion than subjects carrying the plus genotype. Urinary S-PMA excretion correlated with SB ($R = 0.17$) and FPG sites ($R = 0.25$) in MNBC. The correlation between S-PMA and SB was strongest in subjects with NQO1*1/*2 and *2/*2 genotypes ($R = 0.37$), and between S-PMA and FPG sensitive sites in subjects with the GSTP1*B/*B genotype ($R = 0.39$). In conclusion, this study shows that urban air with high levels of benzene and UFP is associated with elevated levels of SB and FPG sites in MNBC, and that NQO1 and GST genes may modulate the effect.

Introduction

Traffic-generated urban air pollution rich in benzene and particulate matter (PM) is a major health problem in many large cities of developing countries because of widespread use of motorbikes and old automobiles for transportation. Vehicles with two-stroke engines are bound to emit large amounts of volatile organic compounds, polyaromatic hydrocarbons (PAH) and ultrafine particles (UFP), especially when using gasoline of poor quality. For example motorcycles with two-stroke engines emitted five times more benzene than four-stroke motorcycles did, depending on both the quality of fuel, type of engine and engine maintenance (1). Also, poorly maintained petrol powered cars emit blue smoke with high content of volatile organic compounds, including benzene (2).

High-dose exposure of benzene has been associated with a number of adverse health effects, including bone marrow depression and myelogenous leukemia in both rodents and humans (3), although epidemiologic evidence does not permit reliable conclusions following human exposure to the low level of benzene that typically is observed in environmental settings. Benzene undergoes hepatic metabolism, generating hydroquinone, phenol and other compounds with the ability of redox cycling, which may cause excess generation of reactive oxygen species (ROS) (4).

In most large cities, PM is an important constituent of urban air pollution, associated with increased mortality and morbidity of a number of prevalent diseases, including cancer (5). Especially the ultrafine fraction of PM of engine exhaust has received increased focus because of toxicological relevance and because it mainly is generated by local traffic whereas long-range transport is a major contributor to particles of larger size. The toxicological mechanism by which PM contributes to an excess risk is thought to be due to both the ability to directly cause generation of ROS and indirectly by causing inflammation (6). There is compelling evidence from animal experimental models that diesel exhaust particles generate oxidative DNA damage in the lung following pulmonary exposure (7–11).

Oxidative stress is typically assessed as elevated levels of oxidized biomolecules, e.g. oxidative DNA damage, which is relevant for carcinogenesis (12). Especially pre-mutagenic base oxidation products such as 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) have been extensively investigated in various humans tissues (13). For biomonitoring purposes, enzymic detection of DNA oxidation products by, e.g. the single cell gel electrophoresis (comet) assay or similar assays is an easier and more feasible approach (14,15). Oxidative DNA damage can be analyzed by the comet assay in mononuclear blood cells (MNBC) as strand breaks (SB) and formamidopyrimidine DNA glycosylase (FPG) sensitive sites. The FPG protein, purified from *Escherichia coli*, recognizes a broad range of oxidized lesions of adenine and guanine, encompassing 8-oxodG, 4,6-diamino-5-formamidopyrimidine and 2,6-diamino-4-hydroxy-5-formamidopyrimidine. These lesions are excised from the DNA strand by the FPG protein, and the level of FPG sensitive sites thus are measured as additional SB. A recent study conducted in Copenhagen found that 8-oxodG was a suitable marker for assessing individual

Abbreviations: FPG, formamidopyrimidine DNA glycosylase; GPX, glutathione peroxidase; GLM, general linear model; GST, glutathione *S*-transferase; LSD, least statistical difference; MNBC, mononuclear blood cells; NQO1, NAD(P)H:quinone oxidoreductase 1; 8-oxodG, 8-oxo-7,8 dihydro-2′-deoxyguanosine; PAH, polyaromatic hydrocarbons; PM, particulate matter; ROS, reactive oxygen species; S-PMA, *S*-phenylmercapturic acid; SB, strand breaks; UFP, ultrafine particles.

exposure to $PM_{2.5}$ (16). Similarly, earlier studies have shown correlation between 8-oxodG and urinary excretion of *S*-phenylmercapturic acid (S-PMA), a metabolite and biomarker of internal dose of benzene (17–20). This effect was modulated by the NAD(P)H:quinone oxidoreductase 1 (NQO1) genotype, which can prevent redox cycling of benzene metabolites.

Previously, we have documented that the urban air in Cotonou city, the capital of the Republic of Benin, contained high levels of benzene (in excess of 70 $\mu g/m^3$), which is markedly higher than 5 $\mu g/m^3$ recommended by WHO (Ayi-Fanou *et al*., unpublished). In a rural village, there were detectable concentrations of benzene in ambient air, and urine samples contained low, albeit detectable, concentrations of S-PMA. The aim of this study was to investigate the effect of high concentration of benzene and UFP in the urban air on the level of oxidative DNA damage in MNBC. To this end, we recruited three groups of subjects living and working in different areas of Cotonou with high ambient air pollution and a rural reference population, thus enabling a large exposure gradient. The widespread local behavior of self-administrated mixing of poor quality oil in gasoline, containing volatile hydrocarbons for two-stroke motorbikes, causes generation of excessive amounts of both UFP and volatile organic compounds, including benzene. Oxidative DNA damage in terms of SB and FPG sensitive sites was analyzed in MNBC. We measured the number of UFP at different locations in order to assess the contribution of these air pollution relevant constituents. Internal dose of benzene was assessed as urinary excretion of S-PMA. In comparison with earlier biomonitoring studies, we anticipated that the benzene exposure would be sufficiently high to allow reliable determination of the interactions between benzene exposure and genetic polymorphisms in relevant antioxidant and metabolism genes on the level of oxidative DNA damage. Effect modulation of air pollution or benzene exposure was investigated as polymorphisms that are associated with altered enzyme activity in glutathione *S*-transferase (GST), glutathione peroxidase (GPX) and NQO1 genes. The gene–environment interaction of polymorphisms in these metabolism and antioxidant defense genes has been investigated in many molecular epidemiology studies with cancer as the primary endpoint (21). These biomarkers of susceptibility also have proved to be of value as effect modifiers of exposure biomarkers in biomonitoring studies of air pollution (22).

Materials and methods

Subjects and sample collection

Healthy male non-smokers were recruited by announcement at a public meeting initiated to communicate the findings of a previous investigation of air pollution in Cotonou. The subjects were recruited into four groups based on residence and occupational setting. One group of subjects was recruited in a small village (Sohon) ~80 km from Cotonou. In Cotonou, three groups of residents were selected to give a wide range of exposure. The three groups are designated: taxi-moto drivers, roadside (consists of subjects living near roads with high intensity of traffic and occupation in Cotonou) and suburban [subjects living and working in a suburb (Godomey) of Cotonou]. The mean age ± SD (number of subjects) were: taxi-moto drivers (36 ± 6, $n = 29$), roadside residents (27 ± 8, $n = 37$), suburban (36 ± 13, $n = 42$) and rural (40 ± 12, $n = 27$). The sampling was carried out at each location and the samples were transported to the laboratory on ice. The sampling took place in January 2003, which is in the middle of the dry season. Subjects also answered a questionnaire related to variables that might influence biomarkers level, e.g. recent illnesses, job type, age and use of medication. The study was evaluated and approved by the Benin Environmental Agency. All subjects signed an informed consent before entering the study.

Each subject delivered a 50 ml spot urine sample that was stored at −20°C until analysis, and 1 ml heparinized venous blood for isolation of MNBC. The venous blood was diluted with 1 ml PBS, and 200 µl Lymphoprep (Nycomed Pharma, Olso, Norway) was added underneath the diluted blood. MNBC were obtained by collection of the cell layer after centrifugation at 1650 *g* for 20 min (4°C), washed twice with cold PBS and centrifuged at 400 *g* for 15 min at 4°C. Most of the supernatant was removed and the pellet was re-suspended in 1 ml cold RPMI1640 (Gibco, Grand Island, NY) media supplemented with 50% fetal bovine serum (Gibco, Grand Island, NY) and 10% dimethylsulfoxide (AppliChem, Darmstadt, Germany). The MNBC samples were stored at −80°C until analysis.

Ambient UFP

The number concentration of ambient UFP was measured continuously in six locations representing the four exposure groups on separate days by a portable condensation particle counter (TSI 3007; St Paul, MN). The apparatus has continuous measurement of the number of particles with 10–1000 nm in diameter (as number of particles per cubic centimeter). At the University of Copenhagen, we have found that the counting efficiency of the TSI 3007 instrument for particles with 40–200 nm in diameter was within ±10% of the efficiency of a reference instrument (TSI 3010; St Paul, MN). The counting efficiency has been reported as linear from 10^3 counts/cm^3 to 3×10^5 counts/cm^3 (23). The measurements were intended to span the period from early morning (before the first rush hour) to late evening (after the last rush hour). Because isopropanol needed to be loaded to the particle counter approximately every 4 h, the datasets consist of interrupted measurements of the length of ~4 h. The measurements in the suburb and the village were shorter because of time of transport to the locations. The particle counter was set to record UFP counts in 10-s periods. For presentation, the data are presented as average over 1 h.

Oxidative DNA damage

The level of SB and FPG sensitive sites in MNBC were analyzed by single cell gel electrophoresis (comet) assay as described previously (24). Briefly, cells were embedded in 0.75% low-melting point agarose (Sigma) on Gelbond films (BioWhittaker Molecular Applications, Rockland, ME), and lysed for a minimum of 1 h at 4°C (2.5 M NaCl, 0.1 M EDTA, 10 mM Tris base, pH = 10, 1% Triton X-100). FPG sensitive sites were detected by incubation of the agarose-embedded nuclei with 1 µg/ml of FPG protein (kindly provided by Dr Andrew Collins, University of Olso) for 45 min at 37°C. The nuclei were subsequently treated in alkaline solution (300 mM NaOH, 1 mM EDTA, pH >13) for 40 min, and electrophoresed in the same solution at 4°C for 20 min, 25 V and 300 mA. The level of DNA damage was expressed as the mean percent fluorescence in the tail (%DNA in the tail) by the Komet 4.0 software system (Kinetic Imaging) in 50 cells. The net level of FPG sensitive sites was obtained as the difference in score between samples incubated with FPG protein and buffer. The level of DNA damage from each subject was analysed in duplicate. In each experiment (corresponding to one electrophoresis) one aliquot of control MNBC sample was included as assay control.

Urinary S-PMA

Determination of urinary S-PMA was carried out as described previously (18). In brief, the samples were thawed and adjusted to pH = 2. *S*-Benzylmercapturic acid (100 ng/ml) was added to the sample as an internal standard. The organic fraction of the urine was extracted with ethylacetate. After centrifugation at 2500 r.p.m. for 10 min, the ethylacetate layer (supernatant) was collected and dried by vacuum. The residue was dissolved in 1.25 M HCl (in methanol) and incubated for 30 min at 40°C. Subsequently the samples were evaporated under a gentle stream of nitrogen at 45°C. The residue was dissolved in dichloromethane and analyzed on a HP 6890 Series Gas Chromatography (GC) system coupled to a HP 5973 Mass Selective Detector. The results are expressed as ratio concentrations of S-PMA and creatinine determined by the Jaffé reaction in urine (µg S-PMA/g creatinine).

GSTM1, GSTP1, GSTT1, GPX and NQO1 genotypes

Genotypes of GPX ($Pro^{198}Leu$), GSTM1 (gene deletion), GSTP1 ($Ile^{105}Val$), GSTT1 (gene deletion) and NQO1 ($Pro^{187}Ser$) were determined as reported previously (18,25).

Statistics

All data were tested for normal distribution using the Shapiro–Wilks test. The groups were also tested for homogeneity of variance with Levene's test ($P < 0.05$). To fulfill the criteria for normality and homogeneity of variance, data on urinary S-PMA excretion was transformed by the natural logarithm with the base of 2.72 (denoted LogS-PMA below). Urine from five subjects did not contain detectable S-PMA and were given the value of 0.05 µg S-PMA/l,

which corresponds to half the detection limit (0.1 μg S-PMA/l). Differences in the distribution of gene polymorphisms between the groups were tested by χ^2-test with α <5% as the significance level. Exposure–gene polymorphism relationships were analyzed by two different models, with group (model 1) and benzene (model 2) as exposure variables. In the statistical analysis, the NQO1*1/*2 and *2/*2 genotypes were combined, and the GPX*1/*2 and *2/*2 genotypes were combined, because of the small number of subjects with homozygous (*2/*2) genotypes in these genes. Statistical analysis of interactions between polymorphisms was not investigated because of insufficient power (i.e. lack of interactions would be due to type II statistical errors).

It is not possible from this study to discriminate directly between the effect of UFP and benzene because the UFP data were obtained on the group level. However, comparisons of the correlation coefficients provide a feasible estimate of the strength of the associations between different exposures and effects in terms of oxidative DNA damage. The correlation coefficients of models encompassing the group as categorical variables represent the contribution of air pollution as a complex mixture of UFP, benzene and other components, whereas statistical models with benzene and polymorphisms as variables represent the contribution of benzene exposure. The difference between these models may be viewed as follows: $R_{group} > R_{LogS\text{-}PMA}$ represents a description of the data where air pollution as a complex mixture provides a better explanation of the results than benzene. The magnitude of contribution of individual components in air pollution or interactions between components cannot be assessed by this approach.

Model 1. Group differences of urinary LogS-PMA excretion, and SB and FPG sensitive sites in MNBC were analyzed by general linear model (GLM) analysis with differences considered statistically significant at $\alpha < 5\%$ level, and post-hoc analysis as least significant difference (LSD) at $\alpha < 5\%$ level. We used GLM analysis on categorized data in order to obtain correlation coefficients (R) for comparison between different statistical models. The gene–environment interaction effect on the SB, FPG and LogS-PMA was investigated by GLM analysis with polymorphisms and group as categorical variables. The models were considered statistically significant at $\alpha < 1.67\%$ because data of polymorphism were evaluated on LogS-PMA, SB and FPG datasets (Bonferroni correction).

Model 2. The relationship between benzene exposure (LogS-PMA) and the level of DNA damage was investigated by GLM analysis with oxidative DNA damage as the dependent variable and LogS-PMA and polymorphisms as continuous and categorical variables, respectively. *P*-values of the GLM analysis were considered statistically significant at α <2.5% level, because data on polymorphisms were used for both SB and FPG sites (Bonferroni correction). Post-hoc analysis of the GLM models included linear regression analysis of LogS-PMA and DNA damage in stratified datasets of single genotypes.

The statistical analysis was performed in Statistica 5.5 for Windows, StatSoft (1997), Tulsa, OK.

Results

Ambient UFP

Measurements of the number concentration of UFP were carried out on five different locations in Cotonou and in a rural village (Figure 1). There was a large gradient in the concentration of UFP measured in the village, suburb, city background and near highly polluted streets. Moreover gradients in the exposure to UFP can also be discerned from the three measurements at the highly polluted streets. The intersection (street) measurement was obtained from a police stand in the middle of the intersection, whereas intersection (balcony) was obtained in first floor level near the intersection. A gradual decrease occurred during the morning of the day, and an increase in the late afternoon/evening, which most probably are due to complex meteorological explanations that include temperature (increasing during the day; increased UFP formation by condensation of aerosols in exhaust pipes) and wind speed (mainly greater wind speed in the late afternoon and evening; less sedimentation of UFP). However, alterations during the day are most pronounced at the most polluted sites, i.e. three small peaks in the measurement at the intersection (08.00–09.00, 13.00–14.00 and 19.00–20.00) coincide with rush hour.

Gene polymorphisms in GSTM1, GSTP1, GSTT1, GPX and NQO1

The distribution of genotypes of the metabolizing enzyme genes is outlined in Table I. There was a slight inhomogeneous distribution of most of the genes, although this was not statistically significant at $\alpha = 5\%$ level. The inhomogeneous distribution differed between the genotypes, e.g. it appears that taxi-moto drivers as a group contained slightly more subjects with the GSTP1*B/*B polymorphism. The GSTT1 genotype was almost equally distributed between the minus and plus genotype in three of the groups, whereas subjects in the suburban group had a 30 (minus) and 70% (plus) distribution. For the NQO1 polymorphism there is not a clear deviation in the

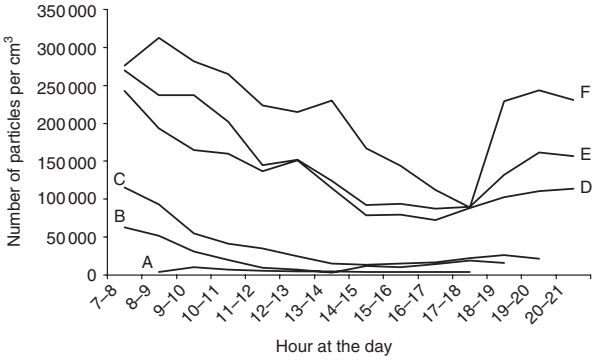

Fig. 1. Concentration of UFP at different locations (as numbers/cm^3). Lines are rural (**A**), suburban (**B**), city background (**C**), intersection on balcony (**D**), traffic circle (**E**) and intersection in the street (**F**). The mean ± SD for a midday hourly sampling with $n = 360$ in each group was: rural, 6961 ± 3223; suburban, 19 980 ± 10 798; city background, 41 230 ± 5822; intersection at balcony, 160 194 ± 56 908); traffic circle, 201 691 ± 49 083; and intersection in the street, 265 145 ± 76 111.

Table I. Distribution of polymorphisms in groups of subjects

Polymorphism	Taxi-moto drivers	Roadside	Suburban	Rural	Total	χ^2-test
GSTT1						5.42
Minus	10	18	12	14	54 (45%)	
Plus	11	15	28	13	67 (55%)	
Missing	8	4	2	0	14	
GSTM1						5.78
Minus	9	5	12	10	36 (30%)	
Plus	12	28	28	17	85 (70%)	
Missing	8	4	2	1	14	
GSTP1						4.6
*A/*A	4	8	8	9	29 (22%)	
*A/*B	11	20	23	12	66 (50%)	
*B/*B	14	5	11	6	36 (27%)	
Missing	0	4	0	0	4	
GPX						0.25
*1/*1	14	16	21	12	63 (49%)	
*1/*2	13	15	17	12	57 (44%)	
*2/*2	2	2	4	1	9 (7%)	
Missing	0	4	0	2	6	
NQO1						5.95
*1/*1	19	22	33	12	86 (65%)	
*1/*2	9	12	8	11	40 (30%)	
*2/*2	1	0	1	4	6 (5%)	
Missing	0	3	0	0	3	

χ^2-tests are not significant at 5% level.

distribution of subjects in one of the groups, except possibly for the distribution between the suburban and rural subjects.

Group effect and gene polymorphism modulation on urinary excretion of S-PMA (Model 1)

The results of urinary LogS-PMA excretion indicated that each of the four groups were statistically significantly different from the others (Figure 2; $P < 0.05$, post-hoc LSD). There was an obvious gradient in LogS-PMA excretion from the village to the most exposed subjects (taxi-moto drivers).

The effect of air pollution on LogS-PMA was investigated by GLM analysis with the group and polymorphisms as categorical variables. This showed single factor effects of the group and GSTT1 genotype (Table II). Stratification of the GSTT1 phenotype indicated that the GSTT1 null genotype was associated with lower (mean = 0.08 μg PMA/g creatinine, $n = 54$) urinary S-PMA excretion as compared with the GSTT1 plus phenotype (mean = 0.15 μg PMA/g creatinine, $n = 67$). Similar analysis of GSTM1, GSTP1, GPX or NQO1 genotypes revealed statistically non-significant interactions and non-significant single factor effects of the polymorphisms, whereas urinary LogS-PMA excretion showed statistically significant single-factor effect ($P < 0.001$, GLM).

Group effect and gene polymorphism on oxidative DNA damage in MNBC (Model 1)

The results of SB and FPG sensitive sites in MNBC are outlined in Figure 3. The mean ± SD of control MNBC samples was 1.35 ± 0.30 and 2.23 ± 0.98 for SB and FPG sensitive sites, respectively ($n = 14$). Simple comparison of differences between the groups revealed a weaker effect on SB ($R = 0.27$, $P < 0.05$, GLM) than on FPG sensitive sites ($R = 0.54$, $P < 0.01$, GLM). The rural population had a lower level of SB than the taxi-moto drivers ($P < 0.05$, post-hoc LSD) and roadside subjects ($P < 0.01$, post-hoc LSD). The taxi-moto drivers had a higher level of FPG sensitive sites than the other groups: roadside ($P < 0.01$, post-hoc LSD), suburban ($P < 0.001$, post-hoc LSD) and rural subjects ($P < 0.001$, post-hoc LSD). There was no significant difference in FPG sites between the roadside and suburban group, whereas both groups had higher levels of FPG sensitive sites than the rural subjects ($P < 0.001$ for both groups, post-hoc LSD).

The effect of air pollution on the SB and FPG was investigated by GLM analysis with polymorphisms and group as categorical variables. This statistical analysis indicated no interaction between single polymorphisms and the group. For the FPG sites, there were single factor effects of the group, whereas polymorphisms had no effect. This indicates that the variation in FPG sites was only explained by difference in exposure between the groups, which could be air pollution. No effect was observed for SB when the analysis included both polymorphism and the group; this is possible due to the lower statistical power when polymorphisms and the group are included as categorical variables.

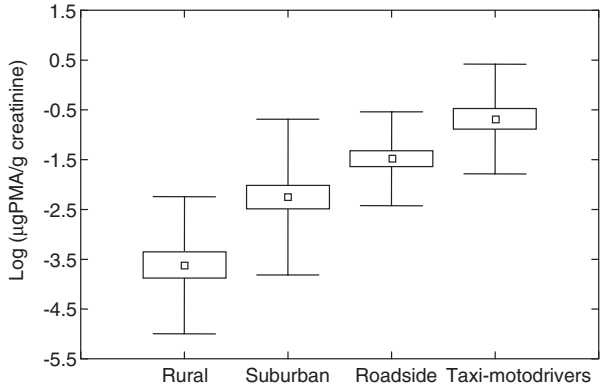

Fig. 2. Urinary S-PMA excretion per creatinine measured by GC-MS. GLM test is statistically significant ($P < 0.001$), and each group is different from the others (post-hoc LSD). Data are expressed as the mean (point) with SEM (boxes) and SD (whiskers).

Table II. Urinary S-PMA excretion stratified by group and GSTT1 polymorphism

Group	GSTT1 polymorphism	
	Minus	Plus
Taxi-moto drivers	0.55 ± 0.45	0.65 ± 0.48
Roadside	0.30 ± 0.38	0.45 ± 0.57
Suburban	0.10 ± 0.11	0.55 ± 1.87
Rural	0.04 ± 0.17	0.16 ± 0.42

GLM analysis for interaction between the group and polymorphism indicate single factor effects of the group ($P < 0.001$) and GSTT1 polymorphism ($P < 0.05$). Data are expressed as the mean ± SD S-PMA excretion (μg S-PMA/g creatinine).

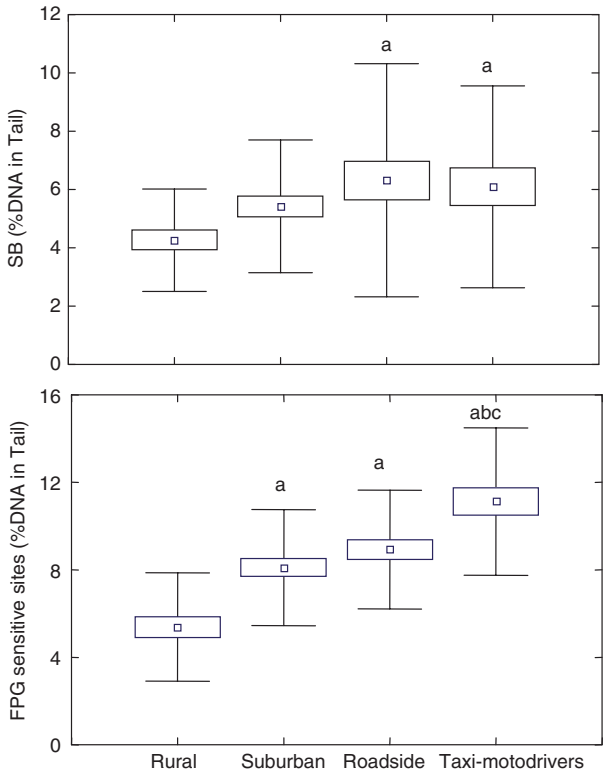

Fig. 3. SB and FPG sensitive sites in MNBC detected by the alkaline comet assay. GLM tests are statistically significant for SB ($P < 0.05$) and FPG sensitive sites ($P < 0.001$). Letters denote post-hoc LSD compared with the rural (a), roadside (b) and suburban (c) group. Data are expressed as the mean (point) with SEM (boxes) and SD (whiskers).

Table III. Correlation coefficients of models describing benzene-induced oxidative DNA damage and effect modification of polymorphism[a]

Polymorphism	*n*	SB	FPG
LogS-PMA	135	0.17	0.25
LogS-PMA*GSTT1	121	0.27	0.27
LogS-PMA*GSTM1	121	0.28	0.25
LogS-PMA*GSTP1	131	0.21	0.37[b]
*A/*A	29		0.15
*A/*B	66		0.18
*B/*B	36		0.39
LogS-PMA*GPX	129	0.21	0.24
LogS-PMA*NQO1	132	0.32[c]	0.25
*1/*1	86	0.04	
*1/*2 and *2/*2	46	0.37	

[a]Analyzed by full factorial GLM test with LogS-PMA as continuous variable and polymorphism as categorical variable (Model 2). Values in the table refer to the correlation coefficient for the models. Models were considered statistically significant at $\alpha = 0.025$ value (Bonferroni correction).
[b]Single factor effect of LogS-PMA and GSTP1 polymorphism ($P < 0.01$). Post-hoc stratification indicates correlation between LogS-PMA and FPG sites in subjects with GSTP1*B/*B genotype.
[c]Interaction between LogS-PMA and NQO1 polymorphism ($P < 0.01$). Post-hoc stratification indicates correlation between LogS-PMA and SB in subjects with NQO1*1/*1 and *2/*2 heterozygote or mutation genotype.

Benzene-exposure and gene polymorphism modulation on oxidative DNA damage in MNBC (Model 2)

Linear regression of SB on LogS-PMA ($R = 0.17$, $P < 0.05$, linear regression) and of FPG on LogS-PMA ($R = 0.25$, $P < 0.01$, linear regression) indicated positive associations between benzene exposure and DNA damage. The correlation coefficients are lower than the corresponding values obtained when using the group in the analysis (0.27 and 0.54 for the SB and FPG sites, respectively), indicating that the group better predicts DNA damage in MNBC than benzene.

The statistical analysis of the interaction between benzene exposure and polymorphism on DNA damage is outlined in Table III. Stratification of the dataset according to genotypes showed an effect of NQO1 polymorphism on the benzene-induced SB, where the effect was entirely due to the *1/*2 and *2/*2 genotype showing a positive correlation ($R = 0.32$, $P < 0.01$, linear regression). For the GSTP1 genotype, there was single factor effect of both LogS-PMA and the polymorphism on the level of FPG sites in MNBC ($P < 0.01$, GLM). However, although non-significant by χ^2-test, there appears to be an over-representation of subjects with the GSTP1*B/*B genotype among the taxi-moto drivers (49 *vs* 15–26% in the other groups), which may hamper direct comparison of differences in FPG sites related to the GSTP1 genotype because of differences in exposure. Stratification of the polymorphism indicated that subjects with GSTP1*B/*B genotype showed a positive correlation ($R = 0.39$, $P < 0.05$, linear regression), whereas correlations were non-significant in the other genotype-based subgroups.

Discussion

This study showed clear-cut elevated levels of SB and oxidative DNA base damage in MNBC of non-smoking subjects with an apparent dose–response relationship according to the gradient of exposure to air pollution from rural to urban areas. The level of oxidative DNA damage correlated with the benzene exposure gradient assessed by urinary S-PMA excretion. The level of ambient UFP also showed an exposure gradient similar to that of benzene exposure. The levels of SB and oxidative DNA base damage in terms of FPG sensitive sites may be caused by different mechanisms, with the NQO1 polymorphism modifying induction of SB, and the GSTP1 polymorphism linked to FPG sensitive sites.

Traffic emission is the most important source of air pollution in Cotonou, Benin, both in terms of polyaromatic hydrocarbons and volatile organic compounds (Ayi-Fanou *et al*., unpublished). There exist no objective measurements of the traffic density in Benin. Compared with Europe and North America, there are many motorbikes and many old cars, and congested traffic is normal in the rush hours. A previous investigation showed that the ambient concentrations of benzene, measured by personal monitors, were 3 and 76 $\mu g/m^3$ for rural referents and taxi-moto drivers, respectively (Ayi-Fanou *et al*., unpublished). Two groups of subjects living near traffic-dense roads were exposed to benzene at concentrations of 48 and 60 $\mu g/m^3$ (Ayi-Fanou *et al*., unpublished). We did not measure the ambient concentration of benzene in the present study, but this can be estimated by the S-PMA excretion because there is a strong correlation between ambient benzene concentration and S-PMA excreted in urine. Using the relationship reported by Ghittori *et al*. (17), the ambient benzene concentration estimated from the urinary S-PMA excretion (mean and 95% confidence intervals) is: 2 (0.9–4), 13 (7–27), 40 (26–62) and 121 (67–219) $\mu g/m^3$ for rural, suburban, cityroad and taxi-moto drivers, respectively. The estimated ambient benzene concentrations are remarkably similar to the levels reported previously. It was not feasible in this study to analyze personal exposure of UFP because the apparatus used for personal sampling of UFP is vulnerable to shakes; this would interfere with the free motion of the subjects because the vehicles are heavily shaken due to the poor condition of many roads in Benin. The ambient concentration of UFP, measured as stationary locations was dependent on the traffic intensity, discerned as increases during rush hours and differences in UFP concentration between different locations. It should be noted that the particle measurements provide no information of the size distribution or toxicity of the UFP measured.

We found a positive correlation between urinary excretion of LogS-PMA and SB in subjects with the *1/*2 and *2/*2 NQO1 genotype, whereas the correlation between LogS-PMA and FPG sites did not depend on the NQO1 genotype. There was no difference in SB between taxi-moto drivers and residents living near polluted roads, although both of these groups had elevated SB compared with the rural subjects. This lack of dose–response relationship at the highest ambient benzene exposure cannot be explained by saturation of the comet assay or different distribution of NQO1 polymorphism. Previously, low-dose urban benzene exposure has indicated a positive correlation between urinary S-PMA excretion and 8-oxodG in lymphocytes of subjects living in Copenhagen, Denmark, whereas no correlation was observed with SB (16). Considering that the level of SB was only slightly elevated in the present study despite the wide benzene exposure gradient, the lack of effect seen in Copenhagen could be due to the exposure being below the detection limit for this biomarker. Similar results have been reported from subjects in Rome, Italy where urban benzene and particle exposure was not associated with differences in SB in MNBC (26). However, gasoline station attendants had higher levels of SB in MNBC than non-benzene exposed referents (27). Correlations have been reported

between *trans*, *trans*-muconic acid (benzene exposure biomarker) and SB, and urinary 1-hydroxypyrene (PAH exposure biomarker) and SB in subjects from areas polluted by lead smelter and waste incinerator plants (28). It is worthwhile to consider that these exposure situations differ from urban air settings and the effect may be due to co-exposure of other air pollution constituents, i.e. benzene may be a proxy-measure of other active components in the environment. Occupational benzene exposure was associated with increased SB in Chinese subjects (29,30). The effect may be more apparent in the Asian population because the susceptibility-associated allele of NQO1 (corresponding to the *2 allele) is more prevalent than among Caucasians. The statistical analysis in this study showed that the elevated level of SB could in part be related to the gene–environment interaction involving the NQO1 since the correlation coefficient increased by incorporation of both benzene and NQO1 in the model ($R_{\text{LogS-PMA}} = 0.17$, $R_{\text{group}} = 0.27$, $R_{\text{LogS-PMA/NQO1}} = 0.32$). NQO1 is a phase II enzyme responsible for the detoxification of quinones, which otherwise may produce ROS by redox cycling. Subjects with the *2/*2 genotype of the NQO1 gene are devoid of hydroquinone-induced enzyme activity, whereas subjects with the *1/*2 genotype have less enzyme activity compared with subjects with the *1/*1 genotype, indicating an inability of subjects with the *2 allele to detoxify benzene metabolites (31). It is probable that excessive amounts of quinone metabolites of benzene generate ROS that ultimately damage DNA by forming SB. The biologic implication of the NQO1 polymorphism can be inferred from observations that subjects with the NQO1*1/*2 genotype appear to be significantly over represented among patients with adult leukemia (32). Also, studies from China have shown that subjects with the NQO1*2/*2 genotype had higher risk of occupationally caused benzene poisoning, although this also depended on concomitant genetic constitution of other metabolic genes such as GSTT1 and CYP2E1 (33,34).

Animal experimental models have shown elevated FPG sites and 8-oxodG in MNBC and bone marrow cells of mice exposed to high dose of benzene (35–37). The present study showed a positive correlation between benzene exposure and FPG sites in MNBC of subjects with GSTP1*B/*B genotype. This is possible through a mechanism of GSTP1-mediated glutathione conjugation of benzene, which could alter the toxicity of some of the DNA damaging reactive intermediates generated from benzene metabolism. However, the highest correlation coefficient was achieved for the model with the group as the only variable, whereas models with LogS-PMA or LogS-PMA/GSTP1 produced lower correlation coefficients ($R_{\text{group}} = 0.54$, $R_{\text{LogS-PMA}} = 0.25$, $R_{\text{LogS-PMA/GSTP1}} = 0.37$). It is probable that other components than benzene in air pollution contribute to the elevated level of FPG sites in the exposed subjects; UFP exposure is a probable variable explaining the variation in FPG sensitive sites. Alternatively, the low correlation coefficient could be because of non-linear genotype–exposure interactions, as has been observed for, e.g. the effect modification of GSTM1 and *N*-acetyltransferase 2 polymorphisms on the level of DNA adducts in PAH-exposed subjects (21). The GSTP1 enzyme is a phase II metabolism protein that is involved in the detoxification of PAH compounds, and most studies of the GSTP1 polymorphisms have concentrated on PAH exposures. Topical application of PAH (7,12-dimethylbenzantracene) has been associated with markedly higher skin cancer incidence in GSTP1 null mice compared with wild-type mice (38), indicating a significant role of GSTP1 in PAH-induced carcinogenesis. In humans, a meta-analysis of eight case-control studies has shown increased odds ratio for lung cancer among subjects having the GSTP1*B/*B phenotype (39). Although associations between GSTP1 polymorphism with other cancer sites have been investigated, the results are conflicting and warrant formal meta-analysis before conclusions should be made. The effect of the polymorphism is not easily interpreted because it appears to cause opposite catalytic efficiency toward planar and non-planar PAH compounds, and this is to some extent dependent on other polymorphisms in the GSTP1 gene (40). To the best of our knowledge, there are no studies published from experimental animal models of PAH-induced FPG sites or 8-oxodG. A recent biomonitoring study showed that subjects with GSTP1*B/*B genotype who smoked had about twice the level of FPG sites in MNBC compared with non-smokers with that genotype, whereas there were no effects of smoking among subjects with GSTP1*A/*A or *A/*B genotype (41). Human PAH-rich exposure circumstances are typically complex with co-exposure from UFP or other constituents that induce oxidative DNA damage.

GSTT1 is normally involved in the detoxification of small molecules. Thus, the association of GSTT1 polymorphism with LogS-PMA excretion indicates that this polymorphism is involved in the glutathione conjugation of benzene. The results support recent results of benzene-exposed Estonian oil shale mine workers where subjects carrying GSTT1 plus genotype had higher urinary S-PMA excretion compared with subjects with the minus genotype (20). Studies of Italian bus drivers and Copenhagen residents, who had lower benzene exposure than observed in the present study, showed no effect of GSTT1 genotype on urinary S-PMA excretion (18,42). Collectively, we can conclude from these data: that GSTT1-mediated glutathione conjugation of benzene with subsequent urinary S-PMA excretion is important across ethnic groups; that the effect is dose-dependent with only effect observed above a moderate ambient benzene concentration. The biological consequence of the GSTT1 polymorphism is difficult to predict because it is involved both in activation and detoxification of environmental carcinogens, and there has been no consistent associations between the GSTT1 genotype and cancer risk (21).

By means of X-ray calibration curves as done by, e.g. the European Standards Committee on Oxidative DNA Damage, the mean ± SD level of FPG modifications per diploid cell with 4×10^{12} dalton DNA can be calculated to 650 ± 160, 1110 ± 188, 1250 ± 198 and 1620 ± 310 for the subjects in the rural, suburb, roadside and taxi-moto driver groups, respectively. These values are similar to other estimations of FPG modifications in lymphocytes, i.e. 870 lesions/cell (43), 1100 lesions/cell (0.28 lesions/10^9 dalton) (44), 1375 lesions/cell (0.23 lesions/10^6 bp) (45), 1440 lesions/cell (0.24 lesions/10^6 bp) (46) and 3500 lesions/cell (1.33 lesions/10^6 bp) (47). Our estimation of FPG lesions also are similar to the consensus of baseline FPG modifications in lymphocytes, i.e. 790–11 100 lesions per diploid cell (assuming consensus of 0.3–4.2 modifications/10^6 dG) (48). These data indicate that the level of oxidative DNA damage in this study are within the range that many laboratories in recent years have reported as the basal level of oxidative DNA damage in MNBC.

Polymorphisms in metabolism or antioxidant enzymes are sparsely investigated in African populations. Presently, it is

difficult to draw firm conclusions of the distribution of most of the genotypes in the African population as has been done for Caucasians, because too few studies have been published to establish reliable meta-analysis. The GSTM1 genotype probably is the most studied polymorphism; the distribution of GSTM1 genotypes in this study was similar to that observed in an African control population, i.e. the frequencies are 27 and 73% for the null and plus genotypes, respectively (49). Also, the predominance of subjects with the homozygous NQO1*1/*1 genotype is similar to that observed (61%) among African-Americans (50). The distribution of GSTP1 genotypes was consistent with distribution among American-Africans [19% (*B/*B), 46% (*A/*B) and 35% (*A/*A) (51)], and east African (Gambia) subjects [21% (*B/*B), 66% (*A/*B) and 13% (*A/*A) (52)] whereas the distribution differed from South and East African populations who had frequencies of 7% (*B/*B), 25% (*A/*B) and 68% (*A/*A) (53).

In conclusion this study showed that subjects living in an urban setting heavily polluted by traffic emissions had high levels of oxidative DNA damage in MNBC. The effect of air pollution clearly was more pronounced on FPG sensitive sites than SB, and the magnitude of effect on these endpoints appeared to be influenced by gene polymorphisms in GSTP1 and NQO1, respectively.

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