Increased urinary excretion of 8-oxo-2′-deoxyguanosine, a biomarker of oxidative DNA damage, in urban bus drivers

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Abstract

Oxidative damage to DNA could be involved in the increased risk of cancer associated with exposure to polluted urban air, which contains a number of oxidants. CYP1A2 is induced by and metabolizes polyaromatic hydrocarbons (PAH) and aromatic amines and could modify effects of exposure to ambient air pollution. Similarly, DNA repair may be influenced by occupational and other exposures as well as modify the effect of DNA damaging agents. As part of a large investigation of the genotoxic burden to diesel exposed workers in transport sectors we studied oxidative DNA damage in 57 non-smoking bus drivers from the greater Copenhagen area. The drivers were studied on a workday and on a day off work. Comparisons were made between drivers from the central (n = 30) and rural/suburban (n = 27) areas of Copenhagen. The rate of oxidative DNA damage was estimated from 24 h urinary excretion of 8-oxo-2′-deoxyguanosine (8-oxoG), a repair product of the highly mutagenic oxidation of guanine in DNA or the cellular pool of GTP. CYP1A2 activity was estimated from the urinary excretion of metabolites of dietary caffeine. The DNA repair was estimated by unscheduled DNA synthesis (UDS) in mononuclear cells isolated on the workday. Repeated measures ANOVA and multifactorial ANCOVA with CYP1A2 activity, age and UDS as covariates were used for statistical evaluation. On the workday, the 8-oxoG excretion was 190 ± 108 and 146 ± 89 pmol/kg 24 h in the bus drivers from central and the suburban/rural areas Copenhagen, respectively (p < 0.05). The 8-oxoG excretion was not significantly different between the workday and the day off. CYP1A2 activity was not affected by driving area but was correlated with the 8-oxoG excretion on the workday (r = 0.53; p < 0.05). UDS was not significantly affected by driving area or correlated with the 8-oxoG excretion. The increased excretion of 8-oxoG in bus drivers from central Copenhagen as compared with drivers from rural/suburban greater Copenhagen suggests that exposure to ambient air pollution causes oxidative damage to DNA. This effect may be modified by the activity of CYP1A2 or a coregulated enzyme. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Oxidative DNA damage; Urban air pollution; 8-Oxo-2′-deoxyguanosine; DNA repair

Abbreviations: PAH, polyaromatic hydrocarbons; 8-oxoG, 8-oxo-2′-deoxyguanosine; UDS, unscheduled DNA synthesis; CYP, cytochrome P450; NAT, N-acetyltransferase; DMS, dimethylsulphate
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1. Introduction

Exposure to urban air pollution has been associated with an increased risk of cancer generally ascribed to DNA adducts from polycyclic hydrocarbons (PAH) and other similar compounds [1,2]. However, urban air pollution also contains a number of oxidants, nitrogen oxides and transition metals. Moreover, the aliphatic and aromatic compounds may induce generation of reactive oxygen species during their metabolism [3]. Resulting oxidative damage to DNA may be important in exposure-related carcinogenesis as the DNA base lesions such as 8-oxo-2'-deoxyguanosine (8-oxodG) are abundant and highly mutagenic [4]. Indeed, short term exposure to traffic pollution in three subjects in Tokyo resulted in an increase in oxidative DNA damage estimated from excretion of 8-oxoguanine, a repair product of this lesion in DNA [5]. Similarly, the excretion of 8-oxodG, the most widely used urinary biomarker of guanine oxidation in DNA, was increased after occupational exposure to benzene and fumes from art glasswork as well as by tobacco smoking [6–11]. The level of 8-oxodG is increased in DNA from central sites of the lung in smokers [12]. In experimental settings diesel exhaust particles and aromatic pollution components such as benzene and benzo[a]pyrene inflict oxidative DNA damage [13–16]. In addition, oxidation of plasma proteins has been shown to be increased in bus drivers from urban as compared with rural greater Copenhagen area [17].

The DNA repair activity can be studied by measuring unscheduled DNA synthesis (UDS) in lymphocytes after standardized DNA insults [18–20]. The repair capacity may be influenced by occupational and other exposures [18] as shown in welders [19] and it may modify the level of damage in DNA, potentially including 8-oxodG.

The aromatic compounds in urban air pollution are metabolized by and induce cytochromes P450 (CYPs) in various tissues, possibly influencing toxic effects [21]. The CYP catalyzed metabolism of foreign compounds such as benzo[a]pyrene can generate reactive oxygen species which subsequently can damage DNA [22,23]. Thus, the activity of CYPs, particularly of the CYP1A subfamily, involved in metabolism of aromatic compounds in air pollution may modify effects in terms of DNA oxidation. The activity of CYP1A2 expressed in the liver can be assessed by urinary metabolites of dietary caffeine and has been shown to be increased by smoking and ingestion of charcoal broiled meat and cruciferous vegetables [24,25]. In addition the urinary caffeine metabolites can be used to estimate the activity of N-acetyltransferase (NAT2) and xanthine oxidase [24], which generate superoxide and could be relevant for oxidative damage [26].

In order to elucidate the health effects and potential cancer risks of occupational exposure to air pollution, mainly in terms of diesel exhaust, a large biomarker study has been carried out in the greater Copenhagen area. The present report concerns oxidative DNA damage and modifying factors in terms of excretion of 8-oxodG, UDS and the activity of CYP1A2, xanthine oxidase and NAT2 in bus drivers from urban and rural/suburban areas of Copenhagen.

2. Material and methods

2.1. Study population

Bus drivers employed by the Copenhagen Company covering the greater Copenhagen area were identified by their employer or trade union [1]. Non-smoking bus drivers were asked to participate in the study after informed consent. The study protocol was approved by the local ethics committee. A total of 107 bus drivers participated in the biomarker studies whereas 57 drivers participated with urine collection in the present study. In order to assess the effect of traffic generated air pollution the drivers were classified by two independent researchers into three groups according to traffic intensity, i.e., city center, suburbs and dormitory/rural areas as previously described [1]. For the analysis in the present study, the suburban and dormitory/rural driving areas were combined in order to have reasonable group sizes. The drivers filled in questionnaires regarding exercise, daily intake of fruits and vegetables, coffee, tea and alcohol and exposure to environmental tobacco
smoke and ambient air pollution during leisure time. The characteristics of the study subjects are summarized in Table 1. There were 33% women in the group driving in the center as compared with 15% women in the group driving in the rural/suburban area of Copenhagen. Otherwise, there were no significant differences between the two groups with respect to the recorded variables in Table 1 or with respect to character and placement in city, suburban or rural surroundings of the home of the subjects (not shown).

The exposure to ambient air pollution in Copenhagen bus drivers was verified by measuring levels of nitrogen oxides by means of passive diffusions samplers in and outside the buses of one line in the city center as well as by fixed stations along the line for 3 weeks [27]. The levels in and outside the bus were identical and corresponded to the levels at fixed stations. The levels were slightly higher than measured at front doors in traffic dense areas of Copenhagen and 5 to 8 times higher than measured at front doors in rural areas. Similarly, in Copenhagen a 6- to 8-fold gradient in benzene, toluene and xylene has been shown between front doors in traffic dense and rural areas [28]. With respect to PAH levels in ambient air in Copenhagen a 2- to 7-fold gradient has been shown from city street to suburbs and villages and even lower values in the open land [29].

2.2. Collection and analysis of samples

Urine samples were collected on a workday and on a day off work. All analytical work was done on coded samples without knowledge of exposure group. For 24 h urine collection one acid prewashed tube was used for each void. Each of the pooled 24 h urine collections was assayed for 8-oxodG as previously described [9] with the exception that 8-oxodG standard for quantification was obtained from Sigma (St. Louis, MO). The 24 h excretion of 8-oxodG was related to body weight. Four to six hours after ingestion of two to four cups of coffee or tea spot urine samples were collected and transferred to tubes with HCl to a pH of around 3.5. These samples were used for the estimation of CYP1A2 and xanthine oxidase activity as well as NAT2 phenotype from caffeine metabolites assayed as previously described [24,30]. The samples were stored at −20°C until analysis. A number of subjects, particularly from the rural/suburban area, failed to collect urine samples, especially on the workday, or had too low caffeine metabolites

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Characteristics of non-smoking bus drivers participating in a study of oxidative DNA damage and urban air pollution in greater Copenhagen</th>
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<tbody>
<tr>
<td></td>
<td>All (n = 57)</td>
</tr>
<tr>
<td><strong>Men/women (n)</strong></td>
<td>43/14</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>45 ± 8</td>
</tr>
<tr>
<td><strong>Body weight (kg)</strong></td>
<td>85 ± 15</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>28 ± 6</td>
</tr>
<tr>
<td><strong>Days off work before sampling</strong></td>
<td>1 [1–5]</td>
</tr>
<tr>
<td><strong>Exposure to air pollution</strong></td>
<td></td>
</tr>
<tr>
<td><strong>During transport (h/day)</strong></td>
<td>0.75 [0.33–1.33]</td>
</tr>
<tr>
<td><strong>During leisure time (h/day)</strong></td>
<td>0 [0–4]</td>
</tr>
<tr>
<td><strong>As tobacco smoke (h/day)</strong></td>
<td>2 [1–4]</td>
</tr>
<tr>
<td><strong>Intake of</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Fruit/vegetables servings/week</strong></td>
<td>18 ± 8</td>
</tr>
<tr>
<td><strong>Coffee or tea (cups/day)</strong></td>
<td>7.2 ± 4.2</td>
</tr>
<tr>
<td><strong>Alcohol (drinks/week)</strong></td>
<td>4 [0–8]</td>
</tr>
<tr>
<td><strong>NAT2 phenotype slow/fast</strong></td>
<td>26/29</td>
</tr>
</tbody>
</table>

BMI, body mass index: the square of the height divided by body weight. Data are frequencies, mean ± SD or median [interquartile range].

* Based on cooking habits and the use of pan content for gravy.
in their urine for reliable analysis. The number of available samples with successful analysis in each group appears from Table 2.

On a workday, blood samples were collected in heparinized tubes and mononuclear cells were isolated for UDS estimation as previously described [20]. UDS was estimated as counts per minute incorporated $^{3}H$-thymidine in the mononuclear cells (200,000) after treatment with dimethylsulphate (DMS 100 μM) or UV (6 J).

2.3. Statistics

The statistical analysis was performed by means of Statistica® for Windows version 5.1 F, StatSoft, 1997, Tulsa, OH. The effect of sex, exposure group, day of investigation and NAT phenotype on log 8-oxodG excretion and CYP1A2 was investigated by multifactorial and repeated measures ANOVA. Potential effect modification on 8-oxodG excretion by continuous variables (age, CYP1A2 ratio, xanthine oxidase, UDS and body mass index) was investigated by ANCOVA. Post hoc, means were compared by the method of least significant differences. Linear regression and correlation analysis were done by the method of least squares. The UDS data were compared between the sexes and exposure groups by the Mann–Whitney U-test. Probability values less than 5% were considered statistically significant.

3. Results

The 24 h urinary excretion of 8-oxodG was significantly higher in the bus drivers from the city center (high exposure group) than in the drivers from the rural/suburban (low exposure group) on both the workday and the day off in repeated measures ANOVA (Table 2). A similarly significant effect was shown in ANCOVA with CYP1A2 activity as covariate and workday/day off and driving area as grouping variables and irrespective of inclusion of sex as a grouping variable in the model. Indeed, 8-oxodG excretion and CYP1A2 activity was significantly correlated in all the bus drivers on the workday ($r = 0.53; p = 0.0001$), irrespective of driving area, whereas there was no correlation on the day off

Table 2
Factors determining urinary excretion of 8-oxodG, CYP1A2 activity and UDS in lymphocytes treated with DMS or UV in 60 bus drivers from central and suburban/rural Copenhagen

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>Men</th>
<th>Women</th>
<th>City center</th>
<th>Rural/suburban area</th>
</tr>
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<tbody>
<tr>
<td><strong>8-oxodG (pmol / kg 24 h)</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Workday</td>
<td>172 ± 102 (49)</td>
<td>182 ± 105 (36)</td>
<td>144 ± 91 (13)$^a$</td>
<td>150 ± 108 (29)</td>
<td>146 ± 89 (20)$^b,c,d$</td>
</tr>
<tr>
<td>Day off</td>
<td>161 ± 110 (51)</td>
<td>176 ± 118 (37)</td>
<td>123 ± 74 (14)$^b$</td>
<td>179 ± 111 (29)</td>
<td>158 ± 106 (23)$^c,d$</td>
</tr>
<tr>
<td><strong>CYP1A2 ratio</strong></td>
<td></td>
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<tr>
<td>Workday</td>
<td>4.2 ± 1.8 (49)</td>
<td>4.4 ± 2.0 (35)</td>
<td>3.7 ± 1.3 (14)$^a$</td>
<td>4.3 ± 2.1 (28)</td>
<td>4.0 ± 1.4 (21)</td>
</tr>
<tr>
<td>Day off</td>
<td>4.3 ± 1.8 (56)</td>
<td>4.8 ± 2.1 (40)</td>
<td>3.2 ± 1.1 (16)$^b$</td>
<td>4.1 ± 2.0 (29)</td>
<td>4.5 ± 2.2 (27)</td>
</tr>
<tr>
<td><strong>Xanthine oxidase</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Workday</td>
<td>0.59 ± 0.07 (49)</td>
<td>0.60 ± 0.05 (35)</td>
<td>0.57 ± 0.09 (14)$^b$</td>
<td>0.58 ± 0.08 (28)</td>
<td>0.60 ± 0.04 (21)</td>
</tr>
<tr>
<td>Day off</td>
<td>0.58 ± 0.08 (56)</td>
<td>0.60 ± 0.08 (40)</td>
<td>0.54 ± 0.10 (16)$^b$</td>
<td>0.58 ± 0.10 (29)</td>
<td>0.58 ± 0.08 (27)</td>
</tr>
<tr>
<td><strong>UDS</strong></td>
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</table>

$^a$ $p < 0.05$ for significant effect of sex in MANOVA.
$^b$ $p < 0.05$ vs. male value.
$^c$ $p < 0.05$ in repeated measures ANOVA and ANCOVA with CYP1A2 ratio as covariate.
$^d$ $p < 0.05$ vs. value in urban group.
Values are mean ± SD or median with upper and lower quartile in brackets; the number of drivers in each group is shown in parenthesis.
Fig. 1. Relationship between urinary excretion of 8-oxodG, a biomarker of oxidative DNA damage, and the activity of CYP1A2 on a workday and a day off work in bus drivers from central (filled circles) and rural/suburban (open circles) Copenhagen. Regression lines with 95% confidence intervals are shown.

\( r = 0.16; p = 0.28 \); Fig. 1). These correlations are significantly different \( (p = 0.049) \). Although the 8-oxodG excretion was lower on the day off than on the workday in both groups of bus drivers the difference was not statistically significant (Table 2). However, most the drivers had been off work for only a few days before sampling (Table 1) and this period was not a significant covariate of the 8-oxodG excretion. The 8-oxodG excretion was 35% higher in men than in women \( (p < 0.05) \) but the difference between the two driving areas were similar (Tables 2 and 3). The 8-oxodG was not significantly correlated with age \( (r = -0.15; p = 0.35) \).

The UDS in mononuclear cells after DMS treatment was higher in women than in men \( (p = 0.047) \) whereas the opposite pattern was seen for UV induced UDS although that difference failed to reach statistical significance \( (p = 0.13; \text{Table 2}) \). There were no significant differences between the bus drivers from the central and suburban/rural Copenhagen with respect to UDS after DMS or UV. The relationship between 8-oxodG excretion and UDS is shown in Fig. 2. The correlation \( (r = 0.26) \) between 8-oxodG and DMS induced UDS was not statistically significant \( (p = 0.08) \). For the UV induced UDS, there was no apparent relationship with 8-oxodG excretion \( (r = -0.13; p = 0.39) \). There was no correlation between the UDS values after the two treatments \( (r = 0.01; p = 0.96) \).

The CYP1A2 caffeine ratio was significantly higher in male than in female bus drivers on the day off whereas the difference was not significant on the

<table>
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<th>Table 3</th>
<th>Urinary excretion of 8-oxodG (pmol/kg 24 h) in male and female bus drivers from central and suburban/rural Copenhagen</th>
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<tbody>
<tr>
<td></td>
<td>Men city center</td>
</tr>
<tr>
<td>Workday</td>
<td>204 ± 110 (20)</td>
</tr>
<tr>
<td>Day off</td>
<td>202 ± 116 (20)</td>
</tr>
</tbody>
</table>

\( *p < 0.05 \) vs. male city center group.

Values are mean ± SD, the number of drivers in each group is shown in parenthesis.
workday (Table 2). There were no significant differences with respect to CYP1A2 ratio between workday or day off in bus drivers or between the high and low exposure group. The xanthine oxidase activity was higher in men than in women but it was not affected by driving area or correlated with the 8-oxodG excretion ($r$-values were below 0.1). The NAT2 phenotype was not evenly distributed between the driving areas although the differences were not significant ($p = 0.28$; chi square test). The 8-oxodG excretion was not related to the NAT2 phenotype (data not shown).

4. Discussion

In the present study, the excretion of 8-oxodG was increased in bus drivers from central Copenhagen as compared with drivers from rural/suburban greater Copenhagen, suggesting that exposure to ambient air pollution causes oxidative damage to DNA. Although there was no effect of driving area on the CYP1A2 ratio, it was correlated with 8-oxodG excretion, particularly on the workday, suggesting that CYP1A2 modifies the oxidative DNA damage inflicted by ambient air pollution. The driving area had no effect on UDS and a relationship with 8-oxodG excretion failed to reach statistical significance, suggesting that DNA repair activity has little influence on this biomarker.

The present increased 8-oxodG excretion in urban bus drivers may partly explain the increased risk of cancer of the lung and other sites in Copenhagen bus drivers as well as other workers exposed to air pollution from motor vehicles [2,31,32]. Indeed, oxidatively modified bases are abundant in DNA from target organs of cancer, including lung, and the levels are increased in tumors and in smokers [4,12,33]. 8-OxodG is among the most abundant of these adducts and probably among the most mutagenic, resulting in G to T transversions in important genes (for review, see Refs. [4,34]). Oxygen radicals can just like PAH adducts induce GT transversions in codon 248/249 of the p53 tumor suppressor gene [35], which represent frequent hot spot mutations in lung and liver cancers [36,37].

In keeping with the present data, smoking and exposure to air pollution from traffic, art glasswork, environmental tobacco smoke or to benzene increase urinary excretion of 8-oxodG or 8-oxoguanine [5–11,38]. Moreover, in animal experiments diesel exhaust particles induce 8-oxodG levels in lung DNA [13,14]. In smokers, both CYP1A2 activity and 8-oxodG excretion are increased [9,24], whereas in accordance with the present data exposure to PAH in
foundry workers did not increase CYP1A2 activity [39]. In the present non-smokers, the 8-oxodG excretion and CYP1A2 activity were correlated particularly under high exposure circumstances. This suggests that urban bus drivers are exposed to air pollution components that are metabolized by CYP1A2 or a similar coregulated enzyme, such as CYP1A1, with generation of reactive oxygen species. Indeed, aromatic pollution components such as benzene and benzo[a]pyrene as well as other procarcinogens, such as heterocyclic amines, inflict oxidative DNA damage in relation to their metabolism in vitro and in animal experiments [3,15,16,22].

The individual exposure was not assessed in the present study. However, in Copenhagen substantial gradients with respect to nitrogen oxides, simple aromatic hydrocarbons and PAH have been demonstrated from city center to suburban and rural areas [28,29,40]. Moreover, at least for nitrogen oxides the levels in and outside a bus are similar [27]. There were no differences between the drivers from the city center and from suburban and rural areas with respect to other sources of exposure to air pollution. Other effects of the exposure to ambient air pollution have been shown in the present study population, e.g., increased PAH adducts [1], chromosomal aberrations and gaps [41] and oxidative damage to plasma proteins [17]. However, there were no significant correlations between the present and those biomarkers on an individual level as discussed elsewhere [42]. As each biomarker represents a specific target, mechanism and/or compartment and the exposure gradients and effects are modest, lack of correlation is not surprising. Moreover, these biomarkers are supplementary and may all be valuable for the assessment of biologically effective doses in exposed populations.

The 35% higher 8-oxodG excretion in men than in women is in accordance with previous studies [9]. Part of this difference may be related to the relatively lower lean body mass in women. There were fewer women in the rural/suburban driving area, which would tend to reduce an increasing effect of driving in the city center on 8-oxodG excretion. However, a similar difference between the two driving areas was seen in men and women, although not significant in the latter, possibly due to the small numbers. Inclusion of gender in the multifactorial analysis of variance did not change statistical significances.

The 8-oxodG excretion was not significantly different between workday and day off in any group of bus drivers. Probably, the work free period of median 1 or 2 days was too short to any change to occur. In a recent smoking cessation study, the 8-oxodG excretion was maximally reduced after 4 weeks [11].

The use of 8-oxodG excretion as a biomarker of the rate of oxidative DNA damage relies on that repair of this lesion in DNA is almost complete and not affected by changes in repair capacity as argued elsewhere [4,43,44]. The minimum accumulation of 8-oxodG in nuclear DNA with age supports that notion [4,43,44]. Thus, the present lack of a significant relationship between 8-oxodG excretion and DNA repair capacity assessed by DMS and UV induced UDS is not surprising. Moreover, the UDS assays estimate the excision repair capacity for alkylated adducts and photoproducts [18–20]. These pathways may not be relevant for repair of 8-oxodG, which is repaired by nucleotide or base excision resulting in 8-oxoG or 8-oxoGua, respectively [45]. Recently, the human 8-oxoGua glycosylase was cloned by several groups [46,47] whereas nucleotide excision repair was shown to contribute to the repair of 8-oxodG in DNA [48]. Urinary 8-oxodG will also come from the highly specific 8-oxodGTP phosphatase (MutT) and 8-oxodGMP nucleotidase enzymes sanitizing the nucleotide pool [49,50]. This prevents incorporation of 8-oxodG in DNA during synthesis, which is more mutagenic than damage in situ [51].

5. Conclusion

The increased excretion of 8-oxodG in bus drivers from central Copenhagen as compared with drivers from suburban and rural/suburban greater Copenhagen suggests that exposure to ambient air pollution causes oxidative damage to DNA. Moreover, this effect may be modified by the activity of CYP1A2 or a coregulated enzyme whereas DNA repair activity assessed by UDS have minimal influence on 8-oxodG excretion and it appears not to be affected by exposure to air pollution.
Acknowledgements

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References


