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Short Communication

Controlled exposure to diesel exhaust and traffic noise – Effects on oxidative stress and activation in mononuclear blood cells

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ABSTRACT

Particulate air pollution increases risk of cancer and cardiopulmonary disease, partly through oxidative stress. Traffic-related noise increases risk of cardiovascular disease and may cause oxidative stress. In this controlled random sequence study, 18 healthy subjects were exposed for 3 h to diesel exhaust (DE) at 276 μg/m^3 from a passenger car or filtered air, with co-exposure to traffic noise at 48 or 75 dB(A). Gene expression markers of inflammation, (*interleukin-8* and *tumor necrosis factor*), oxidative stress (*heme oxygenase* (*decycling-1*)) and DNA repair (*8-oxoguanine DNA glycosylase* (*OGG1*)) were unaltered in peripheral blood mononuclear cells (PBMCs). No significant differences in DNA damage levels, measured by the comet assay, were observed after DE exposure, whereas exposure to high noise levels was associated with significantly increased levels of hOGG1-sensitive sites in PBMCs. Urinary levels of 8-oxo-7,8-dihydro-2′-deoxyguanosine were unaltered. In auxiliary *ex vivo* experiments whole blood was incubated with particles from the exposure chamber for 3 h without effects on DNA damage in PBMCs or intracellular reactive oxygen species production and expression of CD11b and CD62L adhesion molecules in leukocyte subtypes.

Conclusion: 3-h exposure to DE caused no genotoxicity, oxidative stress or inflammation in PBMCs, whereas exposure to noise might cause oxidatively damaged DNA.

1. Introduction

Epidemiologic evidence shows that exposure to ambient air pollution, especially traffic-derived, is associated with cardiopulmonary disease and mortality [1,2]. Moreover, there is sufficient evidence to conclude that exposure to air pollution, particulate matter (PM) in air and diesel exhaust (DE) causes lung cancer [3,4]. Oxidative stress-induced genotoxicity is thought to be an important link to carcinogenesis and has consistently been associated with PM exposure in experimental models [5]. Similarly, elevated levels of biomarkers of oxidative damage to DNA in leukocytes and urine have been associated with exposure to ambient air pollution in relevant populations [6]. DNA damage has been assessed as guanine oxidation products in leukocytes either after DNA extraction, which has an inherent risk of artifacts due to spurious oxidation, or by the comet assay with formamidopyrimidine DNA glycosylase (FPG) for nicking, which is considered free of such potential artifacts [7]. Urinary excretion of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) has also been widely used as a valid biomarker of oxidative stress [8].

Combustion-driven vehicles are highly important sources of both local air pollution and noise. The latter is poorly investigated with respect to oxidative stress endpoints, whereas it is an important risk factor for vascular disease [9]. A study of human subjects exposed to airport noise overnight in their home showed endothelial dysfunction, which was attenuated by administration of the

Abbreviations: CD, cluster of differentiation; DE, diesel exhaust; FPG, formamidopyrimidine DNA glycosylase; hOGG1, human 8-oxoguanine DNA glycosylase; HMOX1, heme oxygenase (decycling-1); IL8, interleukin 8; 8-oxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; PBMCs, peripheral blood mononuclear cells; PM, particulate matter; ROS, reactive oxygen species; SB, strand breaks; TNF, tumor necrosis factor.

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antioxidant vitamin C [10]. Converging lines of evidence indicate that exposure to traffic noise is associated with the risk of breast cancer [11], of which risk factors for the latter could also include oxidative stress and urinary 8-oxodG excretion [12]. Accordingly, traffic-related air pollution and noise not only share sources, but also adverse health effects and these exposures might converge in some of the mechanistic pathways and act synergistically. Yet, little is known in terms of their short-term effects on such potential mechanistic pathways. Short-term effects of controlled exposure to DE have mainly been related to truck engines, whereas the number of diesel powered passenger cars has been increasing rapidly. Nevertheless, effects on oxidative stress and DNA damage have not yet been included in the outcomes as well as potential interactions with concomitant exposure to traffic noise have not been addressed.

The overall aim of this study was to assess the effects of controlled exposure to DE from a light-duty vehicle and traffic noise, each alone and in combination, on biomarkers of oxidative stress, inflammatory response, and DNA damage in both peripheral blood mononuclear cells (PBMCs) and urinary excretion of 8-oxodG. Previous observations from the same study have shown that the DE-exposure resulted in decreased lung function and increased leukocyte counts in peripheral blood [13]. In addition, PM collected in the exposure chamber was assessed for the ability to induce oxidative stress, DNA damage and inflammatory response in human whole blood *ex vivo* in order to simulate effects after potential translocation from the lungs to the circulation.

2. Materials and methods

2.1. Human exposure study

2.1.1. Study population and design

Eighteen subjects (9 male and 9 female, non-smoking and 40–66 years) were recruited for the study as further described in the supplement and previously [13,14]. The study was performed after approval by the Regional Ethical Review Board in Sweden. All the subjects gave their written informed consent. The subjects were exposed 4 times in random sequence inside a specially designed 22 m^3 exposure chamber for 3 h to a reference exposure (filtered air with ~2 $\mu g/m^3$ of PM_1 (<1 μm) and 46 dB(A) traffic noise), DE (276 $\mu g/m^3$ of PM_1 and 46 dB(A) traffic noise), traffic noise (filtered air with ~2 $\mu g/m^3$ and 75 dB(A) traffic noise), and DE + traffic noise (276 $\mu g/m^3$ of PM_1 and 75 dB(A) traffic noise) at rest, sitting relaxed. The traffic noise was recorded at a busy street crossing which was simulated by the high level (75 dB(A)) representing a real-life form of noise.

2.1.2. Collection and analysis of biomarker samples

Peripheral blood samples were collected immediately before and after the exposure. PBMCs were isolated in Vacutainer Cell Preparation Tubes (Vacutainer® CPT Becton Dickinson A/S, Brøndby, Denmark) and stored at −80 °C in preserving media. Urine was collected at three occasions: immediately before and after the exposure, as well as 20 h post-exposure, and analyzed for 8-oxodG.

The levels of DNA strand breaks (SB) and FPG- and human 8-oxoguanine DNA glycosylase (hOGG1)-sensitive sites in PBMCs were measured by the comet assay as further described in the supplement and previously [15]. We usually find substantially higher levels of FPG-sensitive sites than of hOGG1-sensitive sites in human PBMC samples, although the levels of FPG- and hOGG1-sensitive sites are similar and high after treatment with the photosensitizer Ro19-8022 or potassium bromate inducing specifically 8-oxodG [16–18].

The gene expression of inflammation markers (*IL8* and *TNF*), oxidative stress (*HMOX1*), and DNA repair (*OGG1*) were measured in PBMCs by using RT-PCR, as described in a previous study [19]. We selected these 4 genes as most relevant for the mechanisms of action related to inflammation, oxidative stress and possible change in repair of potential DNA damage as seen in a study of controlled exposure to wood smoke [20]. The eight samples collected per subject precluded assessment of a wider transcriptomics profile.

2.2. Ex vivo *study*

For the *ex vivo* tests a sample of the ultrafine (UF < 0.1 μm) fraction of the DEP was collected on a polytetrafluoroethylene filter using the same experimental set-up as in the human exposure study as further explained in the supplement. The particles were extracted with methanol and resuspended in ELGA® water.

Venous blood collected from an anonymous donor was added suspensions of DEP to final exposure concentrations of 0, 2.5, 12.5 and 25 μg/mL with incubation for 3 h in the dark to mimic the duration of the chamber study. After the exposure, whole blood was assigned to assessment of reactive oxygen species (ROS) production by 2′,7′-dichlorofluorescein diacetate and surface adhesion molecules expression in terms of CD62L and CD11b in leukocytes separated in three gates for lymphocytes, monocytes and granulocytes based on forward and side scatter to assess size and granularity by flow cytometry, whereas DNA damage was assessed in PBMCs by the comet assay as described for the human *in vivo* exposure study. The experiment was repeated on 3 different days.

2.3. Statistics

We used linear mixed effects models (*xtmixed*) to evaluate the DE and traffic noise exposure as single factors and the interaction between these two factors for effects on the biomarker measured after the exposure using Stata/IC software (version 13.0). The change with 95% confidence interval related to an exposure was calculated from the regression coefficient in the mixed effects model. The *ex vivo* study data were analyzed using an analysis of variance (ANOVA).

3. Results

3.1. Human exposure study

The concentration of PM_1 in the air was by mass 276 ± 27 $\mu g/m^3$ (mean ± 1 SD). The number size distribution peaked at 89 ± 9 nm (geometric SD: 1.98 ± 0.12 nm), and the mass size distribution at 195 ± 8 nm, determined as described by Wierzbicka and co-authors [14] from mobility number size distribution measured continuously combined with size resolved particle effective density measurements (from DMA-APM). Analysis by transmission electron microscopy revealed that the particles were aggregates, consisting of monomers with a diameter of ~30 nm [14], with the typical aggregate structure of diesel soot. The monomers had the typical microstructure of DEP. The soot aggregates had very little volatile coating (<10% by mass, volatile at 300 °C), and the level of coating was independent of the soot-aggregate size (classified according to the mobility diameter changed stepwise in the range 50–400 nm), indicating that only minor condensation of organics condensed unto the soot structures after the soot-aggregates were formed. The concentration of polycyclic aromatic hydrocarbons (PAHs) in the gas phase was 7.5 ± 0.2 $\mu g/m^3$, with less than 1% of the PAHs in the particle phase (60 ng/m^3) in the air [14]. The PM characteristics and estimated deposited dose to the lung are summarized in Supplemental Table S1.

The results on DNA damage in PBMCs are shown in Table 1. There was no significant interaction between DE and traffic noise