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Oxidative damage to DNA and repair induced by Norwegian wood smoke particles in human A549 and THP-1 cell lines

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ABSTRACT

Genotoxic effects of traffic-generated particulate matter (PM) are well described, whereas little data are available on PM from combustion of biomass and wood, which contributes substantially to air pollution world wide. The aim of this study was to compare the genotoxicity of wood smoke particulate matter (WSPM), authentic traffic-generated particles, mineral PM and standard reference material (SRM2975) of diesel exhaust particles in human A549 lung epithelial and THP-1 monocytic cell lines. DNA damage was measured as strand breaks (SB) and formamidopyrimidine DNA glycosylase (FPG) sites by the comet assay, whereas cell cytotoxicity was determined as lactate dehydrogenase release. The exposure to WSPM generated SB and FPG sites in both cell lines at concentrations from 2.5 or 25 µg/ml, which were not cytotoxic. Compared to all other studied particles, WSPM generated greater responses in terms of both SB and FPG sites. Organic extracts of WSPM and SRM2975 elicited higher levels of SB than native and washed PM at 25 and 100 µg/ml, whereas assay saturation precluded reliable assessment of FPG sites. During a 6 h post-exposure period, in which the medium with PM had been replaced by fresh medium, 60% of the DNA lesions generated by WSPM were removed. In conclusion, WSPM generated more DNA damage than traffic-generated PM per unit mass in human cell lines, possibly due to the high level of polycyclic aromatic hydrocarbons in WSPM. This suggests that exposure to WSPM might be more hazardous than PM collected from vehicle exhaust with respect to development of lung cancer.

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1. Introduction

Combustion of biomass is a considerable source of particulate matter (PM) in air pollution in many regions of the world. Recently, an extensive review concluded that there is sufficient evidence linking exposure to wood smoke with both acute and chronic illness, whereas there is insufficient evidence to conclude whether wood smoke particulate matter (WSPM) is less or more damaging to health, compared to other types of air pollution particles [1]. Indeed, The International Agency for Research on Cancer has concluded that indoor exposure to biomass combustion (mainly wood) is probably carcinogenic for humans [2].

Abbreviations: FPG, formamidopyrimidine DNA glycosylase; LDH, lactate dehydrogenase; PM, particulate matter; PAH, polycyclic aromatic hydrocarbons; ROS, reactive oxygen species; SB, strand breaks; SRM, standard reference material; TSt+, tunnel street particles obtained when studded tires were used; TSt-, tunnel street particles obtained when studded tires were not used; WSPM, wood smoke particulate matter.

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The mechanisms of action behind the adverse health effects of PM are thought to involve the generation of reactive oxygen species (ROS), oxidative stress and inflammation [3]. Among other effects, these responses can give rise to oxidized and mutagenic DNA lesions [4–6]. So far, only few studies have focused on the oxidative stress effects of PM from biomass combustion, but exposure to WSPM has been associated with elevated generation of ROS, release of cytokines, increased levels of DNA strand breaks (SB) and lipid peroxidation products in cell cultures [7–11]. Numerous studies of animals exposed to ambient or diesel exhaust PM by inhalation, instillation or oral gavage have documented increased levels of oxidative damage to DNA in various organs following acute exposure, whereas longer-term exposure is associated with upregulation of the DNA repair system [12–16]. DNA damage has been assessed by the comet assay in several studies on various types of air pollution PM and can be considered as a reliable technique for the detection of particle-induced genotoxicity [5].

Lung epithelial cells are considered to be the primary target tissue of inhaled PM and contribute to ROS formation and oxidative stress. Monocytes have been found to accumulate in the alveoli during lung inflammation and during exposure to PM [17–19]. Monocytes have also been suggested to play a role in particle clear-

ance [20]. In this study two different human cell lines representing alveolar epithelial cells and monocytes were used to compare the genotoxicity and cytotoxicity of WSPM and well-characterized authentic traffic-generated PM with high and low levels of road wear particles due to the use of studded and non-studded tires. Genotoxicity was assessed as SB and oxidative damage to purines in terms of formamidopyrimidine DNA glycosylase (FPG) sensitive sites in DNA by the alkaline comet assay. As a measure of DNA repair we also determined the removal of WSPM induced DNA lesions in the post-exposure period. In addition, we investigated the potential of the organic fraction of WSPM and SRM2975 to generate DNA damage, compared to native and washed PM, to try to elucidate which fraction of the PM that induced the DNA damage.

2. Materials and methods

2.1. Particle material

In this study we have investigated the cytotoxicity and genotoxicity of traffic-generated PM, WSPM, a type of mineral particle, and diesel exhaust PM. The collection procedures and physicochemical characteristics have been described in detail [21]. In brief, WSPM was collected from a conventional Norwegian wood stove during high-temperature combustion of birch. The wood smoke was cooled down by dilution with unfiltered air and the PM was collected on polycarbonate filters, scraped off a total of 25 filters and pooled. The traffic-generated PM samples were sampled in a road tunnel (Oslo, Norway) with a traffic load of 40,000 vehicles/24 h, including cars, trucks and busses fuelled with diesel or gasoline. The samples were collected in two seasons, with or without contribution from cars using studded tires (TSt+ and TSt–, respectively). The PM was sampled continuously for 2 weeks, scraped off the filters and pooled.

To investigate the relative importance of combustion particles and mineral particles from road pavement abrasion in the cytotoxicity and genotoxicity induced by the traffic-derived PM, two reference samples were included; (i) the standard reference material (SRM2975) containing combustion PM from the filtering system of an industrial diesel-powered forklift, purchased from the National Institute of Standards and Technology (Gaithersburg, MD, USA) [22] and (ii) porphyry mineral PM, containing mineral particles only, later referred to as mineral PM (<10 µm, mean particle size of 8 µm) [10,23].

The content of polycyclic aromatic hydrocarbons (PAH) and organic carbon in the different PM samples has been measured and reported previously [10]. The sum of 18 PAH compounds was analysed by gas chromatography–mass spectrometry and the amounts were 73, 381, 9745 and 67 (ng/mg) for TSt+, TSt–, WSPM and SRM2975, respectively. The organic carbon content was determined by thermal optical transmission analysis and the content was 9.3, 24.4, 35.4 and 16.3 (%) for TSt+, TSt–, WSPM and SRM2975, respectively.

2.2. Preparation of particle samples

Fungal spores might be present in WSPM and TSt samples, but were inactivated by methanol treatment. Methanol was added to all the PM samples (5 mg PM/ml methanol) and was suspended by 30 min of sonication. The PM samples were left overnight in the refrigerator. On the next day, the methanol was evaporated under a stream of nitrogen gas and re-suspended in cell medium by sonication in a water bath for 60 min. The PM samples were then vortexed prior to dilution with cell medium to the final concentrations.

To investigate the role of the organic fraction in the DNA damage, the response to equivalent concentrations of native PM, their organic extracts and the washed PM were compared. For organic extraction, samples of WSPM or SRM2975 were suspended in methanol by 30 min sonication in a water bath (0.5 mg PM/ml methanol), and extracted overnight at room temperature. The suspensions were centrifuged for 20 min at 8000 × g to separate the pellet (washed PM) and the supernatant (organic extracts). The washed PM was then extracted a second time in methanol for 4 h. The washed PM and the pooled organic extracts were dried under nitrogen gas, and stored at –20 °C. For cellular exposure, all three fractions (native and washed PM and organic extracts) were suspended in dimethyl sulfoxide (20 mg/ml) and then in cell growth medium (1 mg/ml). The final concentrations of washed PM and organic extracts in the wells corresponded to 2.5, 25 or 100 µg/ml of native PM. The final concentration of dimethyl sulfoxide in wells did not exceed 0.5%.

2.3. Cell cultures

Human lung epithelial A549 and monocytic THP-1 cell lines were obtained from the American Type Culture Collection (Manassas, VA, USA). The A549 cells were grown in F12 nutrient mixture (HAM) supplemented with 10% heat inactivated foetal bovine serum, 1% L-glutamine and 1% penicillin–streptomycin. The THP-1 cells were grown in RPMI 1640 medium supplemented with 10% heat inactivated foetal

bovine serum, 10 mM HEPES, 1 mM sodium pyruvate and 0.1% gentamicin. Both cell lines were incubated at 37 °C in an atmosphere containing 5% CO₂. The cell culture products were obtained from Gibco®, purchased from Invitrogen™, Denmark.

Plates of different sizes were used in the experiments of lactate dehydrogenase (LDH) release and comet assay. Exposures are reported as concentrations (µg/ml). For comparison between the experiments in terms of cell area, the highest concentration (200 µg/ml) in the incubations corresponds to 106 µg/cm² (comet assay) and 133 µg/cm² (LDH assay).

2.4. DNA damage measured by the comet assay

DNA damage was measured as the formation of SB and FPG sites in the DNA by the comet assay as previously described [24,25]. For experiments, 2.25 × 10⁵ cells were seeded into 24-well culture plates (1.9 cm²/well). The A549 cells were seeded 24 h before the exposure because they required time to attach to the wells, whereas the THP-1 cells were diluted to the final cell concentration just prior to the incubation with PM. The cells were treated with 1 ml PM suspensions diluted with cell culture medium to final concentrations; 0, 2.5, 25, 100 and 200 µg/ml. After 3 h of incubation in PM-containing medium, the cells were centrifuged at 3000 rpm for 5 min before addition of melted agarose. In order to detach the A549 cells from the wells, they were treated with trypsin-EDTA (Invitrogen™, Denmark) prior to the centrifugation. The cell culture experiments were carried out on three different days and exposed to PM in duplicates/day ($N_{\text{total}} = 6$).

The role of the organic fraction compared to native and washed PM of WSPM and SRM2975 was only conducted in A549 cells and with PM at final concentrations of 2.5, 25 and 100 µg/ml. The control samples were treated with medium containing 0.5% dimethyl sulfoxide.

The samples were coded before visual scoring, where the level of SB and FPG sites was obtained by scoring 100 nuclei using a five-class scoring system (arbitrary score range: 0–400). The primary comet assay endpoints in arbitrary units were transformed into lesions/10⁶ base pair (bp), using an investigator-specific X-ray calibration curve and assuming that human diploid cells contain 4 × 10¹² Dalton DNA (corresponding to 6 × 10⁹ bp) [26]. The raw data on DNA lesions were baseline-adjusted because the genotoxicity had been analysed in different experiments; the mean of the zero dose for each experiment was subtracted from each data point.

2.5. Removal of DNA lesions measured by the comet assay

The exposure of the cells was carried out as described in Section 2.4, using A549 cells and WSPM at a concentration of 25 µg/ml, because the assessment of removal of DNA lesions requires a high initial level of DNA damage. The cells were exposed to WSPM for 3 h. The medium containing the WSPM was then removed and the cells were washed three times with phosphate buffered saline to remove remaining PM. Fresh medium was added to the cells and they were subsequently incubated 1.5, 3, 4.5 and 6 h. Parallel wells with unexposed cells were harvested at the same time points.

2.6. Cell toxicity

The cytotoxicity of the PM was measured as LDH activity in cell medium by the Cytotoxicity Detection Kit from Roche Applied Science, Penzberg, Germany. An increase in the number of dead or cell membrane-damaged cells increases the LDH activity in the cell culture supernatant. In this assay the well area was 0.3 cm² (96-well culture plates) and the recommended amount of solution was 200 µl in each well. In these experiments the cell cultures were exposed to PM for 24 h because this incubation period is required to obtain reliable measurements of the cytotoxicity.

2.7. Statistics

The LDH data were analysed by the non-parametric Kruskal–Wallis test, with a post hoc Tukey-type multiple comparison test. The difference in genotoxicity between different types and concentrations of PM was assessed by a nested ANOVA analysis with the concentration and type of PM (or extracts) were included as categorical variables. The concentration was nested in the type of PM (Section 3.1). In the comparison of the effects of native and washed PM and organic extracts (Section 3.2), the data were analysed by a hierarchical nested ANOVA with the treatment (native particles, washed particles or extracts) nested in the type of particle (WSPM or SRM2975) and the concentration nested in the treatment. The data on organic extracts were omitted from the statistical analysis of the FPG sites, because they could only be measured by exposure to the lowest dose (2.5 µg/ml). The validity of the nested ANOVA analysis was accepted on the basis of normal distribution of the residuals. Statistically significant effects were accepted at 5% level in the overall nested ANOVA and in the post hoc least significant difference (LSD) tests. The statistical analysis was performed in Statistica 5.5 (StatSoft, Inc., Tulsa, USA).

3. Results

3.1. DNA damage in A549 and THP-1 cells

Fig. 1 depicts the concentration–response relationships of SB and FPG sites in A549 and THP-1 cells. The overall nested ANOVA test showed statistically significant differences between the types of PM. The WSPM generated more SB and FPG sites than the other types of PM in both cell lines ($p < 0.05$; nested ANOVA), except FPG sites generated by TSt+ exposure in A549 cells (Fig. 1B). Although there were statistically significant differences in the genotoxicity between the PM of mineral, TSt–, TSt+ and SRM2975 related to a specific cell type and lesion, there was no consistent evidence that these types of PM differed in their ability of generation DNA damage.

In the A549 cells, there were significantly increased levels of SB after exposure to the PM preparations at 25, 100 and 200 $\mu\text{g/ml}$ ($p < 0.05$; post hoc LSD test), except for TSt+ and mineral PM that only showed significantly increased SB at concentrations of 100 and 200 $\mu\text{g/ml}$ ($p < 0.05$; post hoc LSD test). In the THP-1 cells, there were significantly increased levels of SB at concentrations above 25 $\mu\text{g/ml}$ for TSt– and WSPM ($p < 0.05$; post hoc LSD test), whereas SRM2975 and TSt+ had increased levels of SB at concentrations above 100 $\mu\text{g/ml}$ ($p < 0.05$; post hoc LSD test) and the mineral PM did not shown any significant damage in terms of SB.

The level of FPG sites in A549 cells was increased after exposure to TSt–, TSt+ and WSPM at concentrations above 25 $\mu\text{g/ml}$ ($p < 0.05$; post hoc LSD test), except for the concentration of 100 $\mu\text{g/ml}$ of the TSt– which was only of borderline statistical significance ($p = 0.052$; post hoc LSD test). For WSPM and TSt–, significant levels of FPG sites were found at 2.5 $\mu\text{g/ml}$ in A549 cells ($p < 0.05$; post hoc LSD test). It was impossible to measure the level of FPG sites in A549

cells exposed to 200 $\mu\text{g/ml}$ of WSPM, because the level of SB was very high. This concentration is consequently excluded from Fig. 1B. Exposure to the mineral PM did not generate FPG sites in the A549 cells ($p > 0.05$; post hoc LSD test). In the THP-1 cells, the levels of FPG sites increased for all types of PM, except TSt–, at concentrations above 25 $\mu\text{g/ml}$ (Fig. 1D). The exposure to SRM2975 in the THP-1 cells had a bell-shaped concentration–response curve, in which the level of FPG sites at 200 $\mu\text{g/ml}$ was not statistically significant from the control.

3.2. DNA damage in A549 cells by organic extracts and washed PM

The role of the extractable organic fraction compared to native and washed PM in the generation of SB and FPG sites in A549 cells is presented in Fig. 2. Overall, the induction of both SB and FPG sites were higher in cell cultures treated with WSPM as compared to SRM2975 ($p < 0.05$; nested ANOVA). The native and washed particles had similar ability of SB generation ($p > 0.05$; post hoc LSD test), whereas the organic extract generated a higher level of SB than both the native and washed particles ($p < 0.05$; post hoc LSD test). In general, the generation of DNA damage by the native PM was slightly lower in this experiment compared to the data reported in Fig. 1, which might be due to a ROS scavenging effect of dimethyl sulfoxide.

We were unable to measure FPG sites in cultures exposed to organic extracts of WSPM and SRM2975 at 25 and 100 $\mu\text{g/ml}$, because the level of SB was so high that FPG sites could not be measured reliably. All particle fractions, including the organic extracts, generated FPG sites at the lowest concentration tested (Fig. 2), but there was no statistically significant difference between the level of FPG sites generated by the native and washed particles ($p > 0.05$; post hoc LSD test).

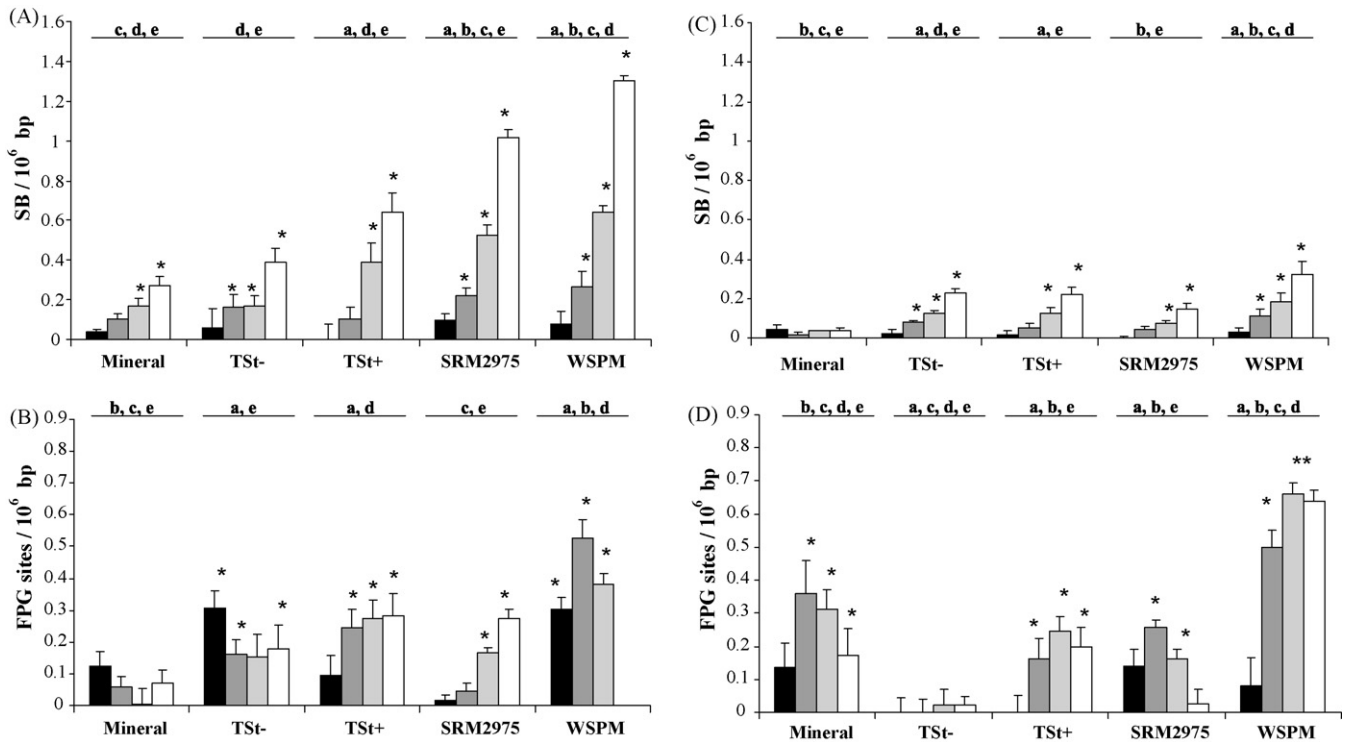


Fig. 1. Baseline-adjusted SB (A) and FPG sites (B) in A549 cell cultures and SB (C) and FPG sites (D) in THP-1 cell cultures exposed for 3 h to PM. Each bar represents the mean \pm S.E.M. ($n = 6$) of cultures exposed to 2.5 (black), 25 (dark grey), 100 (light grey) and 200 (white) $\mu\text{g/ml}$ of PM. The letters above each type of PM indicate statistically significant differences ($p < 0.05$, nested ANOVA) between that and other PM preparations (a, mineral; b, TSt–; c, TSt+; d, SRM2975; e, WSPM). *Statistically significant compared to control ($p < 0.05$; post hoc LSD test).

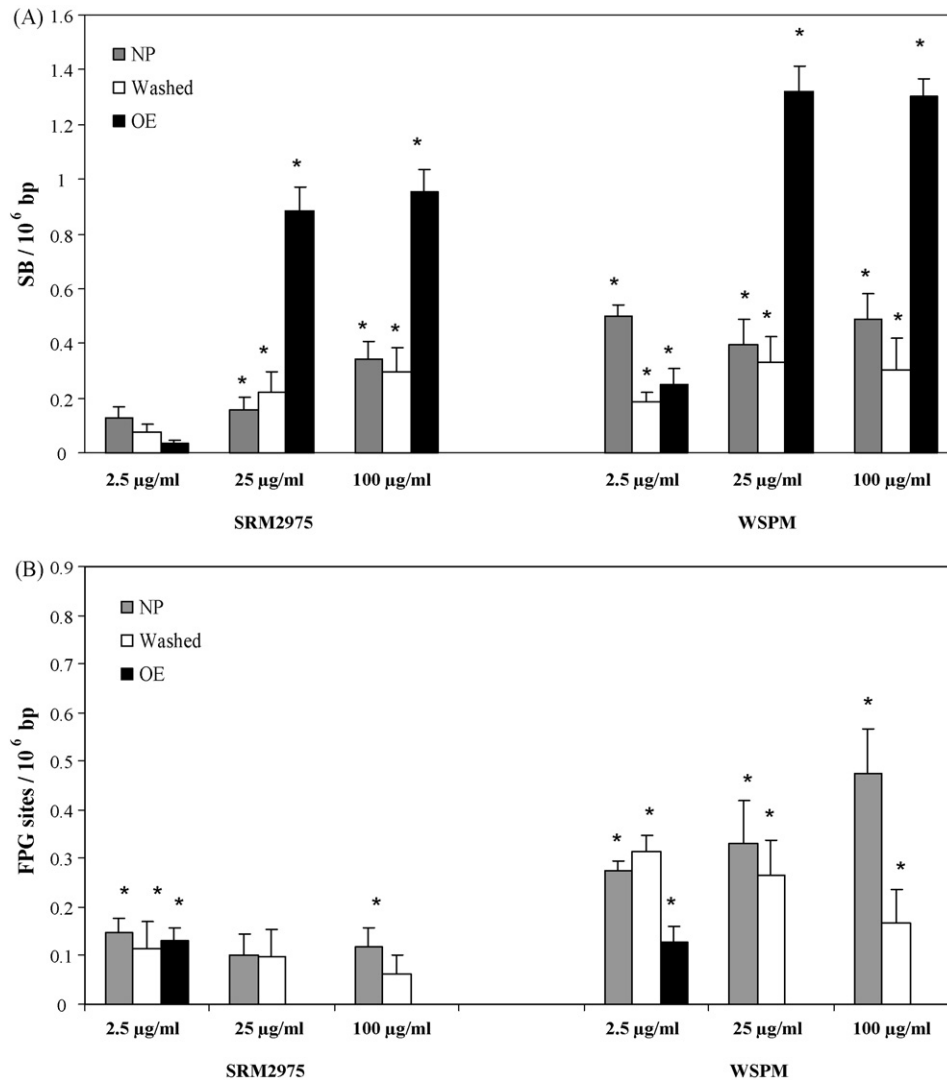


Fig. 2. Baseline-adjusted SB (A) and FPG sites (B) in A549 cell cultures exposed 3 h to native particles (NP), washed particles or organic extract (OE) of SRM2975 and WSPM. Each bar represents the mean \pm S.E.M. ($n=6$). *Statistically significant compared to control ($p < 0.05$; post hoc LSD test).

3.3. Removal of SB and FPG sites in A549 cells exposed to WSPM

To investigate the removal of SB and FPG sites, the A549 cells were incubated with WSPM for 3 h and the medium with the PM was then removed and new medium was added. The results from this experiment are outlined in Fig. 3. The level of lesions is expressed as the fraction of lesions remaining after various post-exposure times. The level of lesions remained almost constant during the first 1.5 h of post-exposure time and then decreased over time, with about 40% of the lesions remaining after 6 h.

3.4. Cytotoxicity of particles and extracts in A549 and THP-1 cells

Fig. 4A and B depicts the cytotoxicity induced by mineral PM, TSt-, TSt+, SRM2975 and WSPM in A549 and THP-1, measured as LDH release (corresponding to the results described in Section 3.1). For the A549 cells, the SRM2975, TSt and WSPM samples increased the LDH release at concentrations of 100 and 200 $\mu\text{g/ml}$, but only statistically significant for some samples and concentrations (Fig. 4A). In the THP-1 cells, only the TSt+ and TSt- PM induced/exhibited statistically significant cytotoxicity at concentrations of 100 and 200 $\mu\text{g/ml}$ ($p < 0.05$; Kruskal–Wallis test).

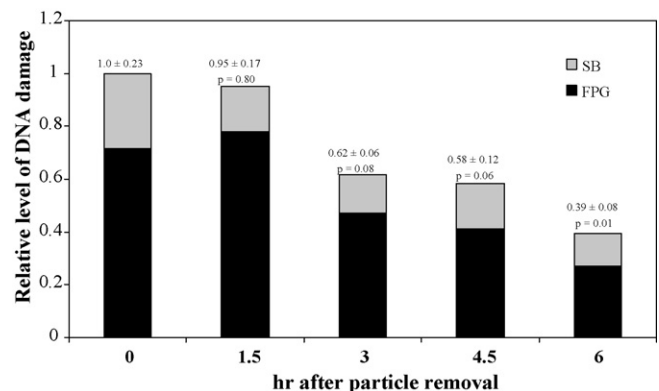


Fig. 3. Removal of SB and FPG sites in A549 cells exposed to 25 $\mu\text{g/ml}$ of WSPM for 3 h (0 h in the figure). The data indicate the fraction of removed lesions relative to the level of lesions after 3 h of exposure (corresponding to 0.15 and 0.37 SB and FPG sites per 10^6 bp, respectively). Each bar represents the mean \pm S.E.M. of four experiments ($n=4$). The p -values represent the statistical significance of all lesions relative to the level of lesions at 0 h (post hoc LSD test).

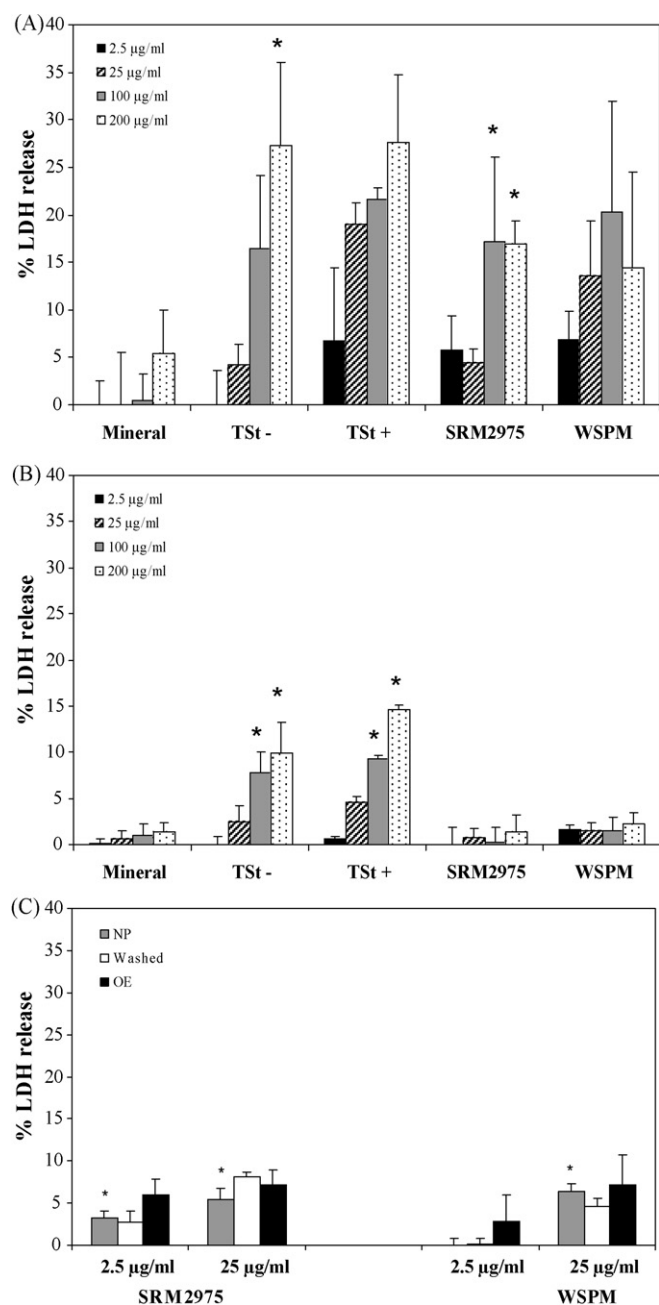


Fig. 4. Cytotoxicity in cell cultures exposed to PM for 24 h. The data in panel A (A549 cells) and B (THP-1 cells) correspond to the exposure and results outlined in Fig. 1, whereas the data in panel C (A549 cells) correspond to the data in Fig. 2. The 100% maximum LDH release is obtained by treatment of cell cultures with Triton X-100, whereas the baseline LDH release in untreated cell cultures is 0%. Bars denote the mean \pm S.E.M. of three experiments ($N_{\text{total}} = 3$). *Statistically significant compared to control ($p < 0.05$; Kruskal–Wallis test).

Fig. 4C depicts the data on cytotoxicity measured as LDH release in A549 cells exposed to organic extracts, native and washed PM of SRM2975 and WSPM (corresponding to the results described in Section 3.2). The native SRM2975 showed significant LDH release at concentrations of 2.5 and 25 $\mu\text{g/ml}$ ($p < 0.05$; Kruskal–Wallis test) and the WSPM at a concentration of 25 $\mu\text{g/ml}$ ($p < 0.05$; Kruskal–Wallis test). The LDH release was not measured at the concentration of 100 $\mu\text{g/ml}$. The cytotoxicity in this experiment was lower compared to that observed in Fig. 4A and 4B. In keeping with the effect observed for the genotoxicity, this could be

due to the presence of dimethyl sulfoxide in the suspension of PM.

4. Discussion

In this study we observed that WSPM elicited DNA damage in terms of SB and FPG sites to a higher extent than other types of PM per unit mass in both lung epithelial and monocytic cell lines. The organic extract of WSPM and SRM2975 generated higher levels of SB than both the native and washed PM, except at the lowest concentration of exposure. We also found that 60% of the DNA lesions induced by WSPM were removed 6 h after the particle suspension was replaced with fresh medium.

The induction of SB followed a concentration-dependent pattern in both human cell lines for most of the PM types, whereas the induction of FPG sites, reflecting oxidative damage to DNA, seemed to reach a plateau at the low concentrations. The significant increase in FPG sites at low concentrations (2.5 and 25 $\mu\text{g/ml}$) was not associated with increased cytotoxicity. This suggests that the detected DNA damage was due to primary genotoxicity elicited directly by the particles in the absence of inflammation, and was not a secondary effect generated during particle-induced inflammation [27]. In general, the WSPM generated more DNA damage than the other types of PM in both A549 and THP-1 cells. However, two to three times higher levels of DNA damage were induced in A549 cells. It should be kept in mind that the adherent cells (A549) might be exposed to higher levels of PM than cells in suspension (THP-1), since the PM sediments during the incubation. This could possibly explain the different levels of genotoxicity in the two cell lines, although the difference may also be a cell type-specific response since the A549 cells are able to engulf PM [28], whereas THP-1 cells to the best of our knowledge do not possess phagocytic activity.

In contrast to the present results, the genotoxicity measured as SB in A549 cells was found to be similar for WSPM and PM collected at a busy street in Stockholm [9]. A few studies have shown increased generation of SB by WSPM exposure [7,9,29], but the present study is the first to investigate the generation of FPG sites by exposure to WSPM in cell cultures. The SB detected by the alkaline comet assay is a measure of genotoxicity, which could arise as a consequence of both oxidative and non-oxidative damage to the DNA as well as transient repair sites. In contrast, the measurement of FPG sites is considered to be more specific towards oxidized purine bases in the DNA. The difference in the concentration–response relationship with a steady increase for SB and a maximum FPG site induction at low exposure concentrations observed presently for most PM in both cell lines, supports this difference in interpretation. Thus, our data show that WSPM generates substantial oxidative damage to the DNA.

WSPM have previously been reported to induce ROS, measured as hydrogen peroxide production in RAW 264.7, but not in A549 cells when DCFH-DA was used for detection [8,29]. In the lung, ROS may be generated directly by the particle surface, by soluble direct or indirect oxidants leached from the PM or as a secondary process by stimulation of target cells or inflammatory cells to produce ROS. The inflammatory potential of the PM used in this study was investigated in THP-1 monocytes. The results suggested that the WSPM had a similar inflammatory potential as the traffic derived PM, only when the strong inflammatory response elicited by endotoxin from the tunnel samples was blocked [10]. Furthermore, the inflammatory response to SRM2975 was negligible compared to that induced by the TSt– and TSt+ PM samples. These data suggest that genotoxicity and inflammatory potential does not correlate well in cell culture experiments, in agreement with Karlsson et al. who reported that street PM and WSPM varied in their ability to induce cytokine release and DNA damage [9].

The WSPM was characterized by a much higher content of organic carbon and PAH than the traffic-related PM (TSt+, TSt– and SRM2975) [10]. Our results indicate that the organic extracts of SRM2975 and WSPM generate more SB than both native and washed PM. FPG sites could only be measured at the lowest concentration of the organic extract (2.5 µg/ml) because the level of SB was very high at the higher concentrations. Both the organic extract and the WSPM itself generated higher levels of SB than the respective SRM2975 preparations, in accordance with the higher levels of organic carbon detected in WSPM [21]. Previous studies comparing the genotoxicity of different fractions of PM reported that native and washed PM and the organic extracts induced similar levels of SB [30,31]. It is not known whether this discrepancy is due to differences in concentrations, extraction procedures, or types of PM. The high genotoxic potential of the organic extracts of SRM2975 and WSPM as compared to their native particles could be due to a higher bioavailability of organic compounds in organic extracts than in native particles. It has been shown that 4 h after a single application of PAH-rich coal tar on the skin there were increased levels of SB in epidermal cells of mice [32]. As shown very recently, PAH are highly capable of inducing both SB and guanine oxidation in A549 cells through ROS generated by the aldo-keto reductase pathway [33].

To our knowledge, this is the first study to investigate the removal of SB and FPG sites following exposure to PM in cell cultures. We used WSPM for this experiment because this type of PM yielded the highest level of FPG sites in A549 cells, whereas the ratio between SB and FPG sites was lower for WSPM as compared to the other types of PM. Usually, cultured cells remove SB very fast (within 1 h) following exposure to clastogens such as ionizing radiation and hydrogen peroxide [34,35]. In experiments using a photosensitizer such as Ro19-8022 and white light, which mainly generates FPG sites, it has been estimated that the half-life of removal of FPG sites is 1–2 h in cultured cells [36,37]. Presently, we observed a half-life of FPG sites of approximately 4 h, but it should be kept in mind that ongoing generation of DNA damage was likely to occur because of incomplete removal of the PM after the exposure period.

The significance of results obtained by in vitro experiments must rely on the fact that they are able to mimic the effects observed in animal experimental models or humans exposed to the same compounds. The comet assay has been used extensively as a biomarker of exposure in biomonitoring studies of genotoxic effects in environmental and occupational settings and can thus serve as a platform of direct comparisons between different test systems [38,39]. In this respect we have previously shown that human exposure to PM from traffic was associated with elevated levels of FPG sites, whereas the level of SB has shown less responsiveness in mononuclear blood cells [40–44]. In healthy humans, wood smoke exposure has been found to increase the levels of inflammation and coagulation markers in plasma and showed possibly lipid peroxidation products measured in urine [45,46]. In the same study, increased levels of *hOGG1* mRNA and decreased levels of SB in mononuclear blood samples several hours after the cessation of the exposure suggested that repair of FPG sites was induced by wood smoke exposure. This was probably due to enhanced repair activity, which was also supported by a slight increase in urinary excretion of 8-oxoguanine repair products.

In conclusion, we have shown that WSPM generates DNA damage both in terms of SB and FPG sites in human lung epithelial and mononuclear blood cell lines, which both represent cell types of the pulmonary target tissue and circulating mononuclear blood cells. The organic fraction of the WSPM induced more DNA damage than the native PM, and WSPM induced SB and FPG sites at lower concentrations than traffic-generated PM. Assuming a sim-

ilar pulmonary deposition of WSPM and traffic-generated PM, it might be anticipated that these types of PM elicit at least similar effects with respect to carcinogenesis. Although these data cannot be extrapolated to humans, the findings do lend support to epidemiological and human inhalation studies which suggest that WSPM may induce adverse health effects.

Conflict of interest

No conflict of interest.

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