Biomarker Alterations Produced in Rat Lung by Intratracheal Instillations of Air Particulate Extracts and Chemoprevention with Oral N-acetylcysteine

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ABSTRACT

Organic matter extracts were obtained from particulates recovered from 10,000-m³ air samples collected in Sicily (Italy). The overall concentrations of acenaphthen, benzo(a)pyrene, phenanthrene, anthracene, fluoranthene, and pyrene were 526 ng/m³ in a highly polluted urban area and 48 ng/m³ in a rural area affected by motor vehicle traffic pollution. After metabolic activation, both samples were mutagenic in Salmonella typhimurium his strains of the TA and YG series, with potencies in TA100 of 140.7 and 11.8 revertants/m³ air, respectively. The samples, resuspended in tricaprylin, were instilled intratracheally in Sprague-Dawley rats for 5 consecutive days, accounting for a cumulative dose in each animal of the organic fractions extracted from 400 m³ air, which corresponds approximately to the volume of air inhaled by a man in 1 month. Treatment with the rural area sample and, at higher levels, with the urban area sample resulted in the formation of adducts to lung DNA, as assessed both by synchronous fluorescence spectrophotometry and by 32P postlabeling, which showed the appearance of up to six individual adducts emerging from diffuse diagonal radioactive zones. The adducts were more efficiently detected by extraction with butanol than by digestion with nuclease P1. DNA binding of air particulate extracts was followed by alterations of early damage biomarkers only in the rats treated with the urban area sample. Repair of DNA damage in lung cells was inferred from a significant stimulation of the nuclear enzyme poly(ADP-ribose) polymerase compared with that in sham-exposed rats. Among the cells recovered by bronchoalveolar lavage, an increase in polymorphonuclear leukocytes and cells of the ciliated respiratory epithelium was accompanied by a relative decrease in pulmonary alveolar macrophages. The frequency of micronuclei was significantly enhanced both in epithelial cells and alveolar macrophages, and binucleated macrophages were also more frequent in treated rats. The thiol N-acetylcysteine, one of the most promising cancer chemopreventive agents, was administered with drinking water to a group of animals receiving the air particulate polycyclic aromatic hydrocarbon fraction from the urban area. N-acetylcysteine prevented or considerably attenuated the alterations of all monitored parameters. These findings provide evidence that, even under outstandingly high exposure conditions, it is possible to protect the respiratory tract from DNA-binding and DNA-damaging air particle carcinogens.

INTRODUCTION

Ambient air in polluted areas contains 2800 identified chemical compounds, many of which are known to possess mutagenic and carcinogenic properties (1). Extracts of polluted air particulates are mutagenic and carcinogenic and induce alterations of intermediate biomarkers in animal models (1). Nevertheless, due to the considerable dilution of carcinogens outdoors and to the high efficiency of the defense mechanisms available in the respiratory tract, the evidence for carcinogenicity in humans living in polluted urban environments is borderline to the sensitivity of analytical epidemiological techniques. Indeed, most studies show a relative risk for lung cancer <1.5 when comparing urban areas with rural areas, yet they suffer from the existence of inevitable confounding factors (2). Similarly, alterations of intermediate biomarkers predictive of carcinogenicity have only been shown to occur in population groups from highly polluted areas (3–5).

PAHs produced by the combustion of fossil fuels constitute one of the most important and most extensively investigated classes of carcinogenic pollutants of ambient air (6). They are predominantly adsorbed onto particles, which, due to the small size (<2.5 μm; Ref. 7), are inhaled and retained in terminal airways.

The present study was undertaken with two main goals. The first was to assess alterations of multiple biomarkers, part of which had never been related to air pollution in earlier studies, in rats receiving i.t. instillations of mutagenic organic fractions extracted from an air particulate. This was collected in a heavily polluted urban area and in a rural area, which was relatively less polluted but affected by motor vehicle traffic. The second goal of the study was to evaluate whether the oral administration of a chemopreventive agent could be successful in protecting the animals from the molecular, biochemical, and cytogenetic damages produced by the massive exposure to air particulate fractions.

The monitored end points included biomarkers evaluating the biologically effective doses of administered compounds, i.e., DNA adducts, as well as early biological effects, i.e., the activity in the lung of the enzyme PARP (EC 2.4.2.30) and the frequency of micronucleated (MN) and binucleated (BN) cells recovered by bronchoalveolar lavage. DNA adducts, which represent a relevant parameter for assessing the potential risk from exposure to polluted air (5), were measured in lung cells by using in parallel SFS and 32P postlabeling with two enrichment procedures. SFS detects DNA adducts of PAH epoxides, especially BPDE (8). 32P postlabeling detects with high sensitivity DNA adducts of various natures, including those formed from polycyclic organic matter present in complex mixtures (9). An increase in endogenous PARP activity provides an early and sensitive signal of primed DNA repair synthesis. PARP activity catalyzes the transfer of ADP-ribose moieties from NADP⁺ to acceptor proteins, which results in a down-regulation of their enzymatic activity (10–12). Although the biological role of this chromatin-bound enzyme has not been thoroughly clarified, PARP has been shown to be involved in the regulation of intrinsic cell functions, including DNA replication and repair, gene expression and rearrangement, cell differentiation, transformation, and proliferation (10–12). Induction of cytogenetic damage was evaluated in PAMs, which represent an ideal cellular target in molecular epidemiological studies with respiratory carcino-
gens (13). Moreover, taking advantage of the fact that a sufficiently high number of epithelial ciliated cells could be recovered by bronchoalveolar lavage, the frequency of the MN form was also measured in this type of cells.

The chemopreventive agent under study was NAC, an analogue and precursor of reduced glutathione, which in a number of studies has been shown to possess a large variety of protective properties and mechanisms toward cancer and possibly other mutation-related chronic degenerative diseases (14–16). Nowadays, NAC is considered one of the most promising cancer chemopreventive agents (17).

MATERIALS AND METHODS

Collection of Air Particulate Samples. Two air particulate samples were collected in September and October 1993 from two sites in or nearby the town of Catania (southeastern Sicily, Italy). One, referred to as an urban area sample, was collected in the center of the town, at a site characterized by heavy motor vehicle traffic. The second sample, referred to as a rural area sample, was collected in the countryside, at a distance of about 150 m from a high-traffic main road.

Samples of particulates were collected by filtering 10,000 m³ air from each of the two sites by using high-volume air samplers (model CTE; Tescora, Milan, Italy) equipped with fiberglass filters having pores of 0.45 μm (Sartorius, Gottingen, Germany), filtering ~10 m³ air/h. The samplers were placed at a height of 1.5 m on the road level. Exposed filters were removed every day, weighed, and replaced with new filters. The weight (normalized at 0°C and 740 mm Hg) of the particulate retained by each filter ranged between 33 and 145 mg/m³ in the urban area and between 11 and 57 mg/m³ in the rural area.

Extraction of Organic Matter and Measurement of PAH Concentration. The organic matter was obtained from each of the two air particulate samples by Soxhlet extraction with cyclohexane (~30 ml/filter) for 8 h, concentrated to a volume of 60 ml in Rotavapor (Buchi, Flawil, Switzerland) at 50°C, and purified by passage through Sephadex LH20.

The solutions were analyzed for the presence of PAHs by using a high-performance liquid chromatography apparatus (821-FP; Varian, Palo Alto, California, USA) equipped with RPC18 reverse-phase columns (Vydac, Varian). The eluting solution was acetonitrile:water, with a flow rate of 1.5 ml/min. Sepharose LH20 was used to separate PAHs. The area ratio of each major PAH peak relative to corresponding internal standards (Società Italiana Chimici, Rome, Italy) was calculated.

Assessment of Mutagenicity of Air Particulate Extracts. The dried organic fraction of each sample was redissolved in 1 ml acetone and diluted 1:20 (v/v) in DMSO. Five doses of each solution, i.e., 100, 50, 25, 12.5, and 6.25 mg/m³ air in the urban area and between 11 and 57 mg/m³ in the rural area.

The extract was evaporated under reduced pressure, redissolved in 5 ml DMSO, and added to 25 ml of the lung lavage fluid to give final concentrations of 0.1, 0.5, 2.0, and 5.0 mg/ml.

PARP Activity in the Lung. Isolated, crude rat lung nuclei were prepared from 1 g tissue as described elsewhere (20). Pelleted nuclei were resuspended at a final concentration of 0.5–1 x 10⁶/ml in modified Merchant’s solution, containing 140 mM NaCl, 2.7 mM KCl, 1.47 mM KH₂PO₄, 0.5 mM EDTA, 8.1 mM Na₂HPO₄, 1 mM DTT, and 0.05 mM phenylmethylsulfonyl fluoride (pH 7.5). Endogenous PARP activity was measured on isolated lung nuclei (5–7 μg DNA) by measuring the incorporation rate of labeled ADP-ribose from [³²P]NAD into acid-insoluble material. The reaction was started by adding the nuclear suspension to 100 μl of a reaction mixture containing 100 mM Tris-HCl (pH 8.0), 10 mM MgCl₂, 10% glycerol, 1 mM DTT, and 100 μM adenosine-2,3'5'cyclic [³²P]NAD (New England Nuclear, Milan, Italy; specific activity, 5 μCi/μmol). Incubation was carried out at 25°C for 4 min. Aliquots of 50 μl were spotted on glass fiber disks and processed as indicated elsewhere (21).

PARP activity was expressed as pmol incorporated labeled NAD/min/μg DNA. The DNA content was determined by a fluorometric micromethod using Hoechst 33258 dye (22).

Purification of Lung DNA and Detection of DNA Adducts. The lungs of the 24 rats were thawed and homogenized in a Potter-Elvehjem apparatus at 4°C in 250 mM sucrose-50 mM Tris-HCl (pH 7.6). DNA was isolated by solvent extraction using an automatic extractor (Genepure 341; Applied Biosystems, Foster City, CA) according to the method of Gupta (23), with some modifications as described previously (24). The extracted DNA was quantified by spectrophotometric analysis. The following A ratios of purified DNA were consistently obtained: 230/260 nm, <0.24; 260/270 nm, <1.20; and 260/280 nm, >1.80 and >1.95.

For SFS analyses, 50-μg DNA aliquots were hydrolyzed in 0.1 M HCl at 90°C for 4 h in sealed glass vials. Synchronous scanning was performed with a fixed λ of 34 nm between excitation and emission, using a Hitachi F-3000 fluorescence spectrophotometer. Peaks in the 379–385-nm emission range were recorded. The intensity of the fluorescence peak was expressed in arbitrary fluorescence units, indicating the difference between the intensity of the signal yielded by the peak and the baseline, which was further subtracted by a fixed value of 3. This was assumed to be an arbitrary threshold of...
Air particulate extracts were devoid of mutagenicity in the absence of a metabolic system (data not shown). In the presence of S9 mix, the rural area extract exhibited a borderline mutagenicity, whereas the urban area extract was strongly mutagenic (Table 1). The mutagenic activity, which could be accurately quantified from dose-response curves, was 11.9-fold higher in the urban area extract than in the rural area sample when tested in S. typhimurium strain TA100, 35.6-fold higher in TA98, and 49.1-fold higher in YG1024. TA100 and its derivatives were more sensitive than TA98 and its derivatives in terms of induced revertants/m³ air. The mutagenic response was not substantially changed in the OAT- and NR-overproducing derivatives, except in YG1024, the OAT-overproducing derivative of TA98, in which the concentration of revertants/m³ air was 2.6-fold higher than in the parental strain. When BP (2 μg/plate) was tested as a positive control in the presence of S9 mix, the ratios of induced revertants: spontaneous revertants were 7.1 ± 0.5 in TA100 (mean ± SD of seven separate experiments), 5.8 ± 0.4 in YG1029, 7.2 ± 0.3 in YG1026, 3.3 ± 0.4 in TA98, 13.9 ± 2.4 in YG1024, 8.1 ± 1.7 in YG1021, and 5.6 ± 0.3 in TA97a. The apparently higher mutagenicity of BP in TA98 derivatives than in TA100 derivatives depends on their lower background of spontaneous revertants.

Adducts to Rat Lung DNA. The i.t. instillations of air particulate PAH extracts resulted in the formation of adducts to the DNA purified from rat lung homogenates, as assessed by means of three different procedures (Table 2). In particular, DNA adduct levels were below the threshold of sensitivity of SFS in sham-exposed control rats, whereas they were detectable by means of this technique in the rats treated with the rural area sample and, at higher levels, in those treated with the urban area sample. Cotreatment with NAC in drinking water significantly inhibited the formation of adducts produced by the latter extract to such an extent that they were no longer detectable by SFS.

Similar indications were provided by 32P-postlabeling analyses, as detailed for total DNA adduct levels in Table 2 and for individual DNA adducts in Table 3. As shown in the image analyses of the autoradiographs reported in Fig. 1, the postlabeling of lung DNA from rats receiving i.t. instillations of air particulate extracts revealed the presence of distinct individual adducts emerging from diffuse diagonal radioactive zones. Adduct 4 comigrated with the major BPDE-N₂-gDNA adduct observed following i.p. administration of BP to rats as well as with an authentic standard of BPDE-N₂-gDNA. Although one of the six individual adducts (adduct 6) could only be detected following digestion with nuclease P1, the levels of all remaining adducts were much higher following extraction with butanol. Thus, as assessed by

### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rural area</th>
<th>Urban area</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAH (ng/m³ air)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>5</td>
<td>26</td>
</tr>
<tr>
<td>BP</td>
<td>6</td>
<td>64</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>15</td>
<td>75</td>
</tr>
<tr>
<td>Anthracene</td>
<td>5</td>
<td>130</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>11</td>
<td>153</td>
</tr>
<tr>
<td>Pyrene</td>
<td>6</td>
<td>78</td>
</tr>
<tr>
<td>S. typhimurium strain</td>
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<td></td>
</tr>
<tr>
<td>TA100</td>
<td>11.8</td>
<td>140.7</td>
</tr>
<tr>
<td>YG1029 (TA100 OAT +)</td>
<td>NT²</td>
<td>168.4</td>
</tr>
<tr>
<td>YG1026 (TA100 NR +)</td>
<td>NT</td>
<td>151.9</td>
</tr>
<tr>
<td>TA98</td>
<td>0.8</td>
<td>28.5</td>
</tr>
<tr>
<td>YG1024 (TA98 OAT +)</td>
<td>1.5</td>
<td>73.7</td>
</tr>
<tr>
<td>YG1021 (TA98 NR +)</td>
<td>NT</td>
<td>29.4</td>
</tr>
<tr>
<td>TA97a</td>
<td>NT</td>
<td>21.8</td>
</tr>
</tbody>
</table>

² The results are expressed as revertants/m³ air, as inferred from the equations of regression lines drawn by testing five doses of air particulate extracts in the presence of the S9 mix.

Results

**Chemical and Mutagenic Characterization of Air Particulate Extracts.**

The concentration of six PAHs was measured in the examined air particulate cyclohexane extracts (Table 1). The total concentration of PAHs in the particulate extract from the urban area (526 ng/m³) was 11 times higher than that from the rural area (48 ng/m³). All individual PAHs had higher concentrations in urban area extracts, with ratios varying between 5 (phenanthrene) and 26 (anthracene).

### Table 2

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Adducts to lung DNA</th>
<th>Controls</th>
<th>Rural area</th>
<th>Urban area</th>
<th>Urban area with NAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFS</td>
<td>&lt;3.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[³²P]Butanol²</td>
<td>0.27 ± 0.45</td>
<td>1.25 ± 0.35</td>
<td>6.24 ± 3.05</td>
<td>2.27 ± 2.66</td>
<td></td>
</tr>
<tr>
<td>[³²P]Nucleic P1²</td>
<td>0.07 ± 0.11</td>
<td>0.14 ± 0.16</td>
<td>0.78 ± 0.91</td>
<td>0.32 ± 0.49</td>
<td></td>
</tr>
<tr>
<td>Lung pADPRP (pmol/mg DNA)</td>
<td>0.58 ± 0.11</td>
<td>0.39 ± 0.17</td>
<td>1.11 ± 0.18</td>
<td>0.83 ± 0.28</td>
<td></td>
</tr>
<tr>
<td>MN Pam (%)</td>
<td>0.83 ± 0.75</td>
<td>1.50 ± 1.05</td>
<td>2.83 ± 2.23</td>
<td>1.33 ± 0.82</td>
<td></td>
</tr>
<tr>
<td>BN Pam (%)</td>
<td>11.83 ± 4.17</td>
<td>12.50 ± 4.76</td>
<td>23.17 ± 4.96</td>
<td>12.33 ± 5.35</td>
<td></td>
</tr>
<tr>
<td>MN epithelial cells (%)</td>
<td>5.00 ± 0.52</td>
<td>0.30 ± 0.64</td>
<td>1.50 ± 0.84</td>
<td>0.50 ± 0.55</td>
<td></td>
</tr>
</tbody>
</table>

² Daily i.t. instillations of tricarprylin (100 μl each) for 5 consecutive days.

### Table 3

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>N-acetylcysteine (NAC)</th>
<th>Controls</th>
<th>Rural area</th>
<th>Urban area</th>
<th>Urban area with NAC</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
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</table>

² Daily i.t. instillations of air particulate extract (8 μl plus 92 μl tricarprylin) for 5 consecutive days, accounting for a cumulative exposure of each rat to the particulate extract from 400 m³ air.

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the Kruskal-Wallis test, the total adduct levels detected following treatment with butanol were significantly higher \( (P < 0.01) \) than those detected following treatment with nuclease P1. As assessed by the Mann-Whitney test, a significant difference \( (P < 0.05) \) between the two adduct enrichment procedures also occurred with two individual spots (spots 1 and 2).

Compared with control samples, total adduct levels in the rural area samples were increased 7-fold (butanol) and 2-fold (nuclease P1), and those in the urban area sample were increased 23-fold (butanol) and 11-fold (nuclease P1). Cotreatment with NAC reduced the total adduct levels 2.7-fold (butanol) and 2.4-fold (nuclease P1; Table 2). In general, similar trends were observed with the individual DNA adducts enriched with butanol (Table 3). The levels of the DNA adducts digested with nuclease P1 were too low to evaluate NAC activity.

**Activity of PARP in the Rat Lung.** The activity of PARP bound to rat lung chromatin was doubled following treatment with the air particulate extract from the urban area. The other two samples were not significantly different from controls (Table 2).

**Cytological and Cytogenetic End Points in Rat Bronchoalveolar Lavage Cells.** The cellular composition of bronchoalveolar lavage was influenced by the treatments of rats (data not shown). In particular, an enhanced presence of ciliated epithelial cells was noted in all treatment groups but especially in the urban area group, in which such an increase was 3-fold, compared with sham-exposed rats. Polymorphonuclear leukocytes were also increased by 37%. These variations were accompanied by a relative decrease of PAMs. The observed cytological alterations are biologically relevant yet not statistically significant, due to marked interindividual variations, although in the case of epithelial cells, the Kruskal-Wallis test approached the threshold of significance \( (P = 0.098) \).

The appreciable number of epithelial cells recovered by bronchoalveolar lavage allowed us to evaluate cytogenetic effects not only in PAMs but also in this type of cells. Cytogenetic data are summarized in Table 2. A slight increase of MN cell frequency in both PAMs and epithelial cells in the rural area sample was not statistically significant. Conversely, the i.t. instillation of the air particulate extract from the urban area produced significant and remarkable variations in the monitored end points, i.e., 3.4-fold and 4.5-fold increases of MN cell frequency in PAMs and epithelial cells, respectively, and a doubling of BN PAMs. In the rats treated with the same air extract but receiving oral NAC, all cytogenetic alterations were prevented, the recorded frequencies being of the same magnitudes as those observed in sham-exposed rats.

**Correlations between Biomarkers.** Several significant correlations between biomarkers were pointed out by evaluating all results obtained with each technique in the 24 rats under study. Thus, the results of SFS correlated with total adduct levels detected by \( 32^P \) postlabeling following enrichment with butanol \( (P < 0.05) \) as well as with the results of BN PAMs \( (P < 0.01) \), MN PAMs, and MN epithelial cells \( (P < 0.05) \). Likewise, total adducts detected following extraction with butanol correlated with BN PAMs \( (P < 0.01) \) and MN epithelial cells \( (P < 0.01) \). All five individual spots detected by this enrichment procedure correlated with BN PAMs, at \( P < 0.001 \) for spots 1 and 3, \( P < 0.01 \) for spot 5, and \( P < 0.05 \) for spots 2 and 4. Spots 3 and 4 additionally correlated with MN epithelial cells \( (P < 0.05) \), and spot 5 correlated with MN PAMs \( (P < 0.01) \). Lung PARP activity correlated with spot 2 (nuclease P1 enrichment) and with MN cell frequency in PAMs \( (P < 0.05) \) and epithelial cells \( (P < 0.01) \). MN epithelial cells correlated with BN PAMs \( (P < 0.01) \).

**DISCUSSION**

The results obtained in the present study provided evidence that the i.t. administration of air particulate organic fractions extracted from polluted environments induced alterations of all investigated intermediate biomarkers in the respiratory tracts of rats. These included formation of adducts to lung DNA as detected by SFS and \( 32^P \) postlabeling with butanol or nuclease P1 enrichment, enhancement of PARP in lung cells, an increase of MN cell frequency in PAMs and cells of the ciliated respiratory epithelium, and an increase of BN PAMs.

Chemical analyses showed that the sample derived from the urban area was very heavily polluted by PAHs, but the rural area sample did also contain appreciable amounts of the same compounds, thus indicating that the collection site in the countryside was affected by its proximity to a high-traffic road. The analytical procedure used was not suitable to detect the presence of nitro-PAHs or arylamines, which typically result from diesel emissions (5). The results of mutagenicity assays showed an absolute requirement for metabolic activation. The urban area sample had the same potency in TA100 and in its NR- or OAT-overproducing derivatives, but its mutagenicity was enhanced in the NR-overproducing derivative of TA98. It is noteworthy that BP, tested as a positive control, exhibited a similar spectrum of sensitivity in the S. typhimurium his \( ^+ \) tester strains, except that the mutagenicity of this PAH was enhanced not only in YG1024, the OAT-overproducing derivative of TA98, but also in YG1021, its NR-reproducing derivative. A similar conclusion was drawn by testing 3-methylcholanthrene.\(^4\) The lack of influence of OAT and NR production on the mutagenicity of PAH in TA100 has been reported already (19, 27) and suggests that metabolic pathways involving these enzymatic activities may contribute to the activation of PAHs to frameshift mutagens reverting the hisD3052 mutation in TA98. In any case, it is remark-

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\(^4\) A. Camoirano and S. De Flora, unpublished data.

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\(^{a}\) Daily i.t. instillations of tricaprylin (100 µl each) for 5 consecutive days. UD, undetectable (<0.01 adducts/10^6 nucleotides).

\(^{b}\) Daily i.t. instillations of air particulate extract (8 µl plus 92 µl tricaprylin) for 5 consecutive days, accounting for a cumulative exposure of each rat to the particulate extract from 400 m³ air.

\(^{c}\) NAC was given with drinking water, at a calculated daily intake of 2 g/kg body weight, starting 24 h before the first i.t. instillation.

\(^{d}\) \( P < 0.05 \) compared to controls, as assessed by means of the Mann-Whitney nonparametric test.

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**Table 3 Detection of individual DNA adduct to rat lung DNA by \( 32^P \) postlabeling (butanol enrichment)**

<table>
<thead>
<tr>
<th>Spot no.</th>
<th>Controls (^{a})</th>
<th>Rural area (^{a})</th>
<th>Urban area (^{a})</th>
<th>Urban area (^{a}) + NAC (^{a})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>UD ( \pm 0.53 )</td>
<td>1.46 ± 0.46(^{d})</td>
<td>0.96 ± 0.60</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.27 ± 0.36</td>
<td>0.73 ± 0.65</td>
<td>2.05 ± 1.28(^{d})</td>
<td>1.22 ± 1.07</td>
</tr>
<tr>
<td>3</td>
<td>UD ( \pm 0.36 )</td>
<td>0.68 ± 0.36</td>
<td>0.03 ± 0.04(^{e})</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>UD ( \pm 0.25 )</td>
<td>1.72 ± 1.42</td>
<td>0.58 ± 0.57</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>UD ( \pm 0.36 )</td>
<td>0.36 ± 0.46</td>
<td>UD</td>
<td></td>
</tr>
</tbody>
</table>

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\(^{a}\) Daily i.t. instillations of tricaprylin (100 µl each) for 5 consecutive days. UD, undetectable (<0.01 adducts/10^6 nucleotides).
able that BP and the air particulate PAH fraction had a similar yet not overlapping spectrum of activity in the bacterial tester strains. This may depend on the presence of undetected PAH derivatives in our sample, on a different spectrum of activity compared with BP of other PAH species contained in the same sample, or on variations of activity occurring in the examined complex mixtures.

Detection of DNA adducts was the most sensitive of the investigated parameters. In fact, this biomarker of biologically effective dose was the only one that was affected by the rural area sample, thus indicating that binding of air particulate extracts to the DNA of the lung mixed cell population in this group of rats was not followed by alterations of early damage biomarkers.

SFS is a relatively simple, inexpensive, and time-saving molecular dosimetry technique. Although not as sensitive as $^{32}$P postlabeling, it is noteworthy that this technique revealed the presence of DNA adducts in the lungs of rats treated with the rural area sample and, at higher levels, in those of rats treated with the urban area sample. SFS detects the adducts formed by BPDE (8) and, to a lesser extent, those formed by ultimate metabolites of other PAH species (28, 29). Cigarette smoking had been found to be the only factor discriminating positivity by SFS of the DNA extracted from human PAMs (30). Among the various cell types populating the respiratory tract, PAMs are the most suitable for the detection of DNA adducts due to their easy recovery by bronchoalveolar lavage and to their intense capacity to phagocytose foreign particles, coupled with their ability to activate PAHs (13, 31). This suggests that SFS can only detect adducts to lung DNA under extreme conditions of exposure to polluted air, as was the case in the present experimental study.

$^{32}$P postlabeling analyses detected diffuse diagonal radioactive zones, which are typically induced by exposure to complex mixtures (32), as well as up to six distinct individual DNA adducts, one of which comigrated with an authentic BPDE-N$_2$-dG reference standard. Of the PAHs that were measured in air particulate extracts, BP (33) and fluoranthene (34) are well known to be genotoxic in a number of short-term tests and react with DNA by forming multiple adducts detectable by $^{32}$P postlabeling. Pyrene has been assayed in a variety of genotoxicity test systems with controversial results (6). It is, in any case, mutagenic in S. typhimurium strains TA97, TA98, and TA100 (35). Most literature data are negative with anthracene and phenanthrene (6), but mutagenicity in S. typhimurium TA97 has been reported (35). Little is known about acenaphthene, which is a mitotic poison (36) but was nonmutagenic in S. typhimurium strains TA1537 and TA1538 (37).

Adduct levels were significantly higher after treatment with butanol than after digestion with nuclease P1. This is consistent with data indicating that, although the relative efficiency of these two enrichment procedures may vary depending on the PAH species (33, 38), DNA adducts derived from complex mixtures occurring in the environment are sensitive to nuclease P1 dephosphorylation (9, 39). In some studies, these patterns have been tentatively ascribed to adduction of nitro-PAHs (5).

All monitored biomarkers reflecting early biological damage were significantly affected by treatment of rats with the air particulate extract from the urban area. The enhancement of endogenous PARP enzyme activity. In parallel, cytological alterations occurred in bronchoalveolar lavage cells, and the frequency of MN cells was enhanced in PAMs and ciliated epithelial cells, along with an increase of BN PAM frequency. The induction of BN cells has been proposed as an index of toxicity and aneuploidy related to complex chromosome and spindle arrangements (40). In general, cytogenetic alterations correlated with the formation of DNA adducts, as detected either by SFS or by $^{32}$P postlabeling following enrichment with butanol.

The dose administered to each animal accounted for the air particulate recovered from 400 m$^3$ air, which corresponds approximately to the average volume of air inhaled by a man in 1 month. It is clear that, due to the reasons mentioned in "Introduction," the conclusions drawn in this experimental study cannot be extrapolated to humans. Yet, it is even more meaningful that, under outstanding conditions of exposure to mixed carcinogens contained in the air of a polluted urban environment, the chemopreventive agent NAC was successful in preventing or considerably attenuating all biomarker alterations. The observed decrease of the molecular dose and inhibition of the subsequent biological damage to lung cells, resulting from the oral administration of NAC, are consistent with previously reported protective effects produced by this thiol in rats treated with individual carcinogens, such as BP and 2-acetylaminofluorene, or with complex mixtures, such as cigarette smoke (14, 24, 41–44). NAC works throughout all stages of the carcinogenesis process, from initiation to invasion and metastasis, via multiple mechanisms (15, 16, 45). The chemopreventive activity of NAC mainly results from the nucleophilic and antioxidant properties of its molecule in the extracellular environment, as well as from its ability to act as a precursor of intracellular reduced glutathione, thereby triggering all defense mechanisms associated with this tripeptide (15, 16). The administration of pharmacological doses of this drug to humans decreases the excretion of smoke-related mutagens in urine (16). NAC is currently under clinical testing in a Phase III cancer chemoprevention trial (46) and in several Phase II studies evaluating the modulation of intermediate biomarkers (17).

REFERENCES


Biomarker Alterations Produced in Rat Lung by Intratracheal Instillations of Air Particulate Extracts and Chemoprevention with Oral N-acetylcysteine

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