Effects of Induction and Age-dependent Enzyme Expression on Lung Bioavailability, Metabolism, and DNA Binding of Urban Air Particulate-absorbed Benzo[a]pyrene, 2-Nitrofluorenone, and 3-Amino-1,4-dimethyl-5H-pyridol-(4,3)-indole

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The effect of interactions between urban air particulates (UAP) and carcinogens on bioavailability, metabolism, and DNA binding was studied in the isolated perfused and ventilated rat lung. The availability of benzo[a]pyrene (BaP) varied from 29 to 60% after intratracheal doses of carcinogen particulates dissolving extremely slow and fast, respectively. Several cytochrome P450 enzyme (P450) inducers acting as 2,3,7,8-tetrachlorodibenzo-[p]dioxin-receptor ligands have been identified in UAP extracts. β-Naphthoflavone (BNF) was used to study how P450 induction alters the lung metabolism of carcinogens. Pretreatment increased the lung clearance for BaP 8-fold and for 2-nitrofluorenone (2NF) by a factor of four from 0.55 ± 0.06 ml/min to 2.37 ± 0.62 ml/min. Studies with the intact lung and with isolated lung cells show that carcinogen metabolism and pharmacokinetics depend both on the route of exposure and dosage and on the distribution of specific enzymes. A cytochrome P450II1B1 enzyme was detected in lung epithelial cells where it catalyzes 9-hydroxylation of 2NF. This rat lung 2NF-9-hydroxylation capacity increases in parallel with the age-dependent up-regulation of lung P450II1B1 expression. Both human and rat lung tissue have the capacity to form 9-hydroxy-2-nitrofluorenone (9-OH-2NF) that is mutagenic. A BNF-inducible P450IA1 was detected in endothelial and alveolar type II cells. Consequently, aromatic hydroxylation dominated when 2NF was dosed directly into the lung circulation. Pretreatment of rats with BNF before intratracheal BaP dosage induced lung cytochrome P450IA1. The 7.8-dihydroxy-9,10-oxynbenzo[a]pyrene-deoxyguanosine adduct and the total lung DNA adduct levels increased significantly from a peak level of 75 ± 8 to 151 ± 19 fmole/mg DNA in lungs from control and BNF pretreated rats, respectively. 3-Amino-1,4-dimethyl-5H-pyridol-4(3H)-indole (TRP-P1) is a potent mutagen and carcinogen identified in UAP extracts. Dietary BNF pretreatment of rats altered the 14C-TRP-P1 distribution as analyzed by whole-body autoradiography. An enhanced retention was observed in the small intestine, forestomach, esophagus, and lung. UAP catalyzes oxygen radical formation and deoxoguanosine-8-hydroxylation, which were inhibited when the UAP samples were extracted with organic solvents or when they were incubated in the presence of desferroxamine. We therefore postulate that a polycyclic aromatic hydrocarbon autooxidation pathway may be responsible for generation of hydrogen peroxide, which may be further converted to hydroxyl radicals through an iron-dependent reaction. — Environ Health Perspect 102(Suppl 4):147–156 (1994).

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Introduction

A complex mixture of carcinogens, enzyme inducers, and metals are present in extracts from urban air particulates (UAP) and several interactions may occur after UAP deposition.

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mutation caused by unrepaird lesions, or DNA adducts formed between reactive carcinogen metabolites and DNA. Results indicating a positive correlation between enzyme inducibility or enzyme expression to carcinogen activation and DNA adducts have been published (16–18). Some conclusions point toward a positive correlation between exposure levels, metabolic capacity, DNA adducts, and cancer risk; however, contradictory results exist (19,20).

Other factors such as different capacity to repair DNA lesions also may modulate the carcinogenic effect of carcinogens, which is evident in individuals with limited DNA repair capacity and an increased risk for developing cancer (21,22).

In addition to DNA damage caused by PAH metabolites, reactive oxygen species also may mediate particular associated toxicity (23–25). Oxygen radicals have been implicated in DNA adduct formation (26,27), in mutagenicity (28), and in carcinogenesis (29,30). It has been shown that tumor-promoting asbestos and other PM catalyze formation of hydroxyl radicals (OH) and superoxide anions (O2−) (25). Auto-oxidation of PAH and quinone generation has been suggested as another mechanism through which such reactive oxygen species may be formed (32). PAH bound to UAP may thus generate DNA-damaging oxygen radicals in parallel with the metabolic cytochrome P450-dependent PAH activation processes mentioned above. In addition, active oxygen species also may contribute to the second oxidative step, the epoxidation, in PAH-diol-epoxide formation. It is possible that oxygen radicals thereby promote the formation of DNA-bound carcinogens when PAH is metabolized in the presence of an oxygen radical generating system such as urban air particulates or asbestos fibers. A separate study was designed to investigate this hypothesis using authentic UAP samples as an oxygen radical generating system.

Materials and Methods

Animals

Male 350 g Sprague-Dawley rats were obtained from ALAB (Sweden) and were given free access to food and water unless otherwise stated.

Chemicals

[G-3H]-Benzo[a]pyrene (B[a]P), specific activity 34 Ci/m mole, and unlabeled B[a]P was obtained from Amersham (Arlington Heights, IL) and from Sigma (St. Louis, MO), respectively. Metabolite standards B[a]P-9,10-diol, B[a]P-4,5-diol, B[a]P-7,8-diol, 9-hydroxy B[a]P, and 3-hydroxy-B[a]P were obtained from IIT Research Institute (Chicago, IL). The metabolite 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene-[1,3-3H] (chemical repository no. 312/567-4318, lot no. CR10-12-2), was obtained from the National Cancer Institute Chemical Carcinogens Reference Standard Repository (NCI, Bethesda, MD). [14C]-Deoxyguanosine-5'-monophosphate, specific activity of 500 mCi/m mole (CPF 89, batch 13), was obtained from Amersham. Hydroxylapatite (DNA grade, Bio-Gel, HTP) was obtained from Bio-Rad Laboratories (Richmond, CA). Calf thymus DNA, deoxyribonuclease I, proteinase K (type XI), alkaline phosphatase (type III), and phosphodiesterase (type II) were purchased from Sigma.

Urban Air Particulates

UAP samples were collected in Gothenburg, Sweden (33) and were characterized as presented in previous studies (33–36). The particulate samples are presented in the text by abbreviations for UAP1, collected in summer in a traffic tunnel; UAP2, collected in the autumn in a rural area outside Gothenburg; UAP3, collected in winter in a traffic tunnel; and UAP4, collected in winter at the street level.

Intratracheal Dosage

Three micrograms B[a]P was administered intratracheally with a Hamilton syringe positioned at the bifurcation controlled by X-ray monitoring during the operation. B[a]P was administered as a suspension with the carcinogen adsorbed onto UAP with the carcinogen dissolved in an artificial alveolar surfactant: dipalmitoyl phosphatidylcholine/phosphatidylglycerol (80:20, w/w) and 1 mg albumin/12 mg phospholipid.

Analyses of 2-Nitrofluorone and Metabolites

Analyses of 2-nitrofluorone (2NF) and its metabolites was performed using a Laboratory Data Control (LDC, Rivera Beach, FL) high-pressure liquid chromatography (HPLC) system, equipped with a Zorbax, C8 column (DuPont, Wilmington, DE) (37).

DNA Isolation and Digestion

DNA was isolated from lung and liver homogenates using chloroform/isoamylicohol/phenol/ether extraction and hydroxylapatite chromatography (36). DNA was quantified by UV spectrophotometry and DNA-bound [3H]B[a]P was quantified by liquid scintillation counting.

Analysis of Benzo[a]pyrene-DNA Adducts

Lung and liver DNA from 5 to 10 animals was digested enzymatically using deoxyribonuclease (DNase) I, phosphodiesterase type II and alkaline phosphatase type III (38). Released nucleoside DNA adducts were purified by Sephadex LH-20 chromatography and analyzed by HPLC on a C18 Radial Pak column connected in line with a liquid scintillation detector. The HPLC column was eluted with a gradient from 42/58 vol/vol-% methyl hydroxide/water (MeOH/H2O) for 15 min, continued with a linear gradient for 60 min to 50/50 vol/vol-% MeOH/H2O. Standard DNA adducts were prepared by incubating 9-hydroxybenzo[a]pyrene (9-OH-B[a]P), [14C]deoxyguanosine, and rat liver microsomes and by reacting anti-B[a]P-7,8-diol-9,10-epoxide with [14C]deoxyguanosine (36).

Analysis of mRNA

RNA extracts were prepared from whole tissues or from isolated cell fractions according to the method presented by Chomczynski et al. (39). Expression of specific P450 mRNAs was then analyzed with use of specific CDNA probes including a) a 758-bp clone corresponding to the 3'-noncoding region of cytochrome P450IA1 mRNA, b) a 265-bp clone corresponding to the 3'-noncoding region of cytochrome P450IA2 mRNA (40), and c) a CDNA probe corresponding to the 211-carboxyl-terminal amino acids of P450IB1 (41). A β-actin CDNA probe was obtained from Cleveland et al. (42). Equal amounts (15 μg) of heat and formamide denaturated RNA were loaded on 1.1% agarose gels containing 25% formaldehyde and electrophoresed and transferred to nylon filters in 20×SSPE (1×SSPE = 180 mM sodium chloride, 10 mM sodium phosphate, pH 7.7, 1 mM EDTA). Filters were then prehybridized at 42°C for 12 hr in a buffer containing 40% formamide, 5 × SSPE, 0.1% sodium dodecyl phosphate, 0.5 mg/ml yeast tRNA, and 2 × Denhardt’s solution. Filter hybridization was performed for 24 hr at 42°C in a hybridization buffer containing 1 to 2 × 106 cp/ml of cDNA probe labeled with deoxyctydine 5′-triphosphate-[32P] using a Multiprime labeling kit (Amersham, England). After hybridization, filters were washed and autoradiographed.

Cell Separation by Elutriation

Lung cells were prepared as presented by Devereux et al. (43,44) using a JE-6B rotor equipped with a standard elutriation chamber. Cell preparations for metabolism studies
were performed as described by Jones et al. (45). Type II alveolar cells were identified by a modified Papanicolaou staining method (46). Nonciliated bronchiolar (Clara) cells were identified by nitroblue tetrazolium staining (47).

Flow Cytometry Measurements
Isolated cells were stained with mithramycin or with propidium iodide for DNA quantification and with fluorescein isothiocyanate for measurement of protein content. The DNA and protein content was then recorded on a laboratory built flow cytometer (48), and the DNA histograms were evaluated using a multichannel analyzer. The 2 × N (2N, diploid) amount of DNA was determined as presented elsewhere (49). The channel number, corresponding to the mean protein content per cell, was calculated using a computer program (50).

High-Pressure Liquid Chromatography Analysis of 8-Hydroxydeoxyguanosine

Hydroxylated deoxyguanosine was analyzed using an HPLC system equipped with a C18 column (51). The UV detector was set at 254 nm and was connected in line with an electrochemical detector (Linear liquid chromatography-304) set in the oxidative mode at + 0.600 V. In analysis of DNA oxidation, the ratio between 8-hydroxydeoxyguanosine (8-OHdG) and deoxyguanosine (dG) was used to express the degree of DNA damage. Standard 8-OHdG was synthesized in the laboratory (52).

2-Nitroflourene Metabolism in Human Lung Tissue and Rat Lung Cells
A total number of 2 × 10^6 rat lung cells was incubated with [14C]2NF (1 μM) for 60 min at 37°C. Incubations with 0.5 g/ml human lung tissue was added 0.5 nmole [14C]2NF. Tissue for control incubations was denaturated by boiling for 10 min in water. In a separate series of incubations, whole rat and human lung tissue surgical resectate (normal tissue and tumor, adenocarcinoma) were dissociated mechanically and incubated with 2NF. Incubations with the three different tissues were performed with or without preincubation with the P4501B1 and P4501A1 inhibitors, and proadifen and α-naphthoflavone, respectively.

Results and Discussion

Availability of Particulate-absorbed Benzo[a]pyrene

The isolated perfused and ventilated rat lung (IPL) was used to investigate interactions between particulates and chemical carcinogen with metabolism, carcinogen bioavailability, and DNA binding as the end point. Release of B[a]P and rate of uptake into the lung perfusion buffer differed significantly between lungs administered with different UAP samples. Lungs dosed with UAP1-adsorbed B[a]P released the carcinogen into the circulation with an absorption half-life of 99 min. In contrast, lungs dosed with microcrystalline, very fast dissolving B[a]P, released the carcinogen with an absorption half-life of 14 min (35,36). Lungs dosed with particulates releasing B[a]P very fast released five times the level of metabolites that was detected in per-
fusate from lung tissues dosed with the UAP1. This was believed to be caused by release rate differences, because UAP1 releases B[a]P extremely slow. When the B[a]P dose was increased from 3 to 100 μg, only a 2-fold difference remained, however, in metabolite generation. This dose-dependent metabolism was interpreted as an effect of enzyme saturation at high B[a]P doses (34). Because the metabolism and absorption processes are parallel, we postulate that the carcinogen release or dissolution rate differences also affect the carcinogen-DNA binding. If a slow rate of carcinogen dissolution avoids saturation of the metabolism, more of the lung-deposited B[a]P should be expected to react on lung DNA (36). To evaluate this hypothesis, a convolution-deconvolution algorithm was used to predict differences in B[a]P bioavailability from differences in in vitro carcinogen desorption. UAP1-adsorbed B[a]P was calculated thus to produce an area under the concentration versus time curve (AUC) in the lung perfusion buffer of 7 ± 2 (pmole/ml/min). The corresponding experimental AUC value reached 10 ± 4 (pmole/ml/min) (36). When B[a]P was administered in an artificial alveolar surfactant, the carcinogen was released from the lung into the circulation with a maximal absorption rate and to an extent of over 80% at the 3-μg dose. In contrast, UAP1-adsorbed B[a]P was released into the lung circulation with less than 20% of the dose. We conclude that the UAP is able to potentiate the lung B[a]P dose by at least a factor of four (36). When the level of total lung B[a]P-DNA binding was analyzed in these lungs, the differences in DNA adduct levels were in the same range (36).

To assess if bioavailability and DNA-binding differences also appear in vivo, UAP-adsorbed B[a]P was dosed to rats by intratracheal instillation and DNA adduct levels were analyzed until 8 weeks after dosage. The highest level of lung DNA adducts, 8 weeks after dosage, was observed in animals dosed with the UAP1 sample, releasing B[a]P very slowly (Figure 1). In the analysis of adduct identity, it was observed that high levels of the carcinogenic 7,8-dihydroxy-9,10-oxynbenzo[a]pyrene-3,4-dioxynosine (7,8-dihydroxy-9,10-oxynbenzo[a]pyrene-3,4-di-Gen) adduct was present in lung DNA from animals dosed UAP with medium and fast B[a]P dissolution rates (Figure 2).

**Figure 4.** Levels of total lung and liver benzo(a)pyrene B[a]P-DNA adducts (fmole/mg DNA) were analyzed for a period of 8 weeks after a single intratracheal dosage of B[a]P. The carcinogen was administered 24-hr after intraperitoneal pretreatment with the 2,3,7,8-tetrachlorodibenzo(p)odioxin receptor ligand B-naphthoflavone (BNF) dissolved in corn oil or after injection of corn oil only. Levels of DNA adducts in control rat lungs are represented with square symbols. Lung DNA adduct levels in BNF-pretreated rats are shown as filled squares, while DNA adduct levels in BNF-pretreated rats is presented with open symbols. Liver DNA adduct levels are represented with open and filled circles in analogy with the presentation of lung DNA adduct levels. Each presented data point is based on individual DNA analysis, with the mean value presented for DNA adduct levels determined in 5 to 10 animals. The mean value is plotted ± SD.

**Enzyme Induction and DNA Adduct Formation**

Induction of carcinogen-metabolizing P450 enzymes (33) is mediated by a UAP-associated factor. This induction is likely to constitute an important co-carcinogenic factor caused by compounds with high affinity for the 2,3,7,8-tetrachlorodibenzo(p)odioxin (TCDD) receptor. Several compounds of this class have been identified in urban air and diesel exhaust particulate extracts (54). It has, in fact, been demonstrated that the rat lung is significantly more sensitive to P450 induction than other tissues (55) and that DNA damage localizes preferentially to lung tissue even after topical application of carcinogens (36). We observed that formation of rat lung carcinogen-DNA adducts increased after pretreatment with the TCDD receptor ligand β-naphthoflavone (BNF). Similar results were observed in isolated perfused and ventilated rat lungs dosed with B[a]P and 2NF. The relationship between P450-enzyme induction and carcinogen-DNA binding was therefore studied further. Interestingly, the lung induction response, measured as cytochrome P4501A1 mRNA levels, did remain elevated for more than 4 days after one intraperitoneal BNF dose. This was significantly different from the induction response in the liver, where the P4501A1 mRNA levels peaked and started to decline already between 1 to 2 days after dosage.
(manuscript in preparation). In contrast to the induction of both P450IA1 and P450IA2 in the liver, no P450IA2 mRNA could be detected in the rat lung (Figure 3). We believe that a biphasic lung P450IA1 mRNA response reflects P450 enzyme induction in different lung cell categories.

The impact of BNF pretreatment on P450 enzyme induction and on levels of covalently DNA-bound B[a]P was analyzed in both lung and liver DNA. BNF pretreatment significantly increased the peak level of total lung DNA adducts from 75 ± 8 fmole/mg DNA to 151 ± 19 fmole/mg DNA (p<0.05, n = 8 - 10). The lung DNA adduct peak time also was delayed from 3 days in control rats to 1 week after B[a]P dosage in rats pretreated with BNF (Figure 4). The levels of the total lung DNA adducts remained significantly higher for 8 weeks in BNF pretreated animals compared to the adduct levels in control rats. A slight, although not statistically significant, decrease was detected in liver DNA-B[a]P levels after BNF pretreatment (Figure 4). Analogous observations also have been made by others who have stated that flavones appear to have opposing effects in the lung and liver with respect to metabolic activation of B[a]P. In fact, studies on the effects of the flavone BNF on human B[a]P metabolism and epoxidation recently has been published with results supporting our conclusion (53).

DNA adducts were analyzed by HPLC, as shown in Figure 5. Figure 5A,B shows elution of [14C]-labeled DNA adducts used as HPLC standards. When DNA from BNF-treated rats was analyzed, we identified a major lung DNA adduct as the carcinogenic metabolite 7,8-diOH-9,10-oxy-B[a]P bound to deoxyguanosine, 7,8-diOH-9,10-oxy-dGuo. One other prominent DNA adduct present in lung DNA from control rats was tentatively identified as 9-OH-4,5-oxy-B[a]P-dGuo. The relative proportion between these two DNA adducts differed depending on both BNF pretreatment and on the time interval from the time of B[a]P dosage until sacrifice. In DNA obtained from control rat lungs, the 9-OH-4,5-oxy-dGuo-DNA adduct dominated 1 week after intratracheal B[a]P dosage (Figure 5C). In contrast, 3 weeks later the 9-OH-4,5-oxy-dGuo adduct and the 7,8-diOH-9,10-oxy-dGuo adducts were present in approximately equal amounts (Figure 5D). The effect of BNF and P450IA1 enzyme induction also was studied 1 week after B[a]P dosage when the 7,8-diOH-9,10-oxy-dGuo-DNA adduct represented more than 90% of the lung DNA adduct level (Figure 5E).

Interestingly, 3 weeks later 40% of the adduct level was identified again as 9-OH-4,5-oxy-dGuo (Figure 5F). This transient shift in DNA adduct identity may reflect the transient P450IA1 induction response because the level of lung P450IA1, catalyzing 9,10- and 7,8-oxidation of B[a]P, prevails only for a limited period of time after a single BNF dose. If lung-deposited B[a]P is retained longer in the lung, a proportion of the carcinogen dose still may be available for metabolism when the enzyme distribution has returned to a low-P450IA1 level with a relatively high level of P450IIB1 (53). The relative DNA adduct retention times are slightly different in Figures 5E,F from the retention times in other panels because of technical reasons. Specific adducts were identified, however, from the retention time of [14C]-labeled 9-OH-4,5-oxy-dGuo, used as standard marker as indicated with an arrow.

Analysis of lung DNA shows that lungs from animals dosed with UAP with slow
Figure 6. Concentration versus time curves for 2-nitrofluorene (2NF) in the isolated perfused and ventilated rat lung. The logarithmic 2NF concentrations versus time profiles were plotted after analysis of the concentration of unmetabolized [14C]2NF in the recirculating lung perfusion buffer. The carcinogen [14C]2NF was dosed either directly to the perfusion buffer (A) or dosed through intratracheal instillation (B). The carcinogen was dissolved in an artificial surfactant solution, composed as presented in "Materials and Methods," to be readily available in the lung. A 50-µg dose of [14C]2NF was administered. Lungs used in the isolated perfused and ventilated lung studies were obtained from control rats, dosed corn oil only (in the figure marked control), and from rats injected with β-naphthoflavone (in the figure marked BNF) 24 hr prior to sacrifice. Each figure shows two representative [14C]2NF perfusion-buffer concentration curves obtained after intratracheal dosage or after dosage directly into the recirculating lung perfusion buffer.

Figure 7. Representative two-dimensional flow cytometry analyses on rat lung cell fractions prepared by counterflow elutriation centrifugation containing parenchymal cells. Alveolar macrophages were eliminated prior to tissue digestion and elutriation by repeated bronchoalveolar lavages. (A) Analysis of the fraction with the highest numerical density of alveolar type II cells. (B) Analysis of the fraction with the highest numerical density of nonciliated bronchiolar (Clara) cells. The histological typing of cells was performed after selective staining, as described in "Materials and Methods." The symbol G2 in the figure marks out the DNA peak representing type II cells in the G2 phase of cell division, while the symbol S is added to mark alveolar type II cells in the DNA synthesis phase. The symbol CC marks Clara cell peak. T-IIC marks out the peak representing alveolar type II cells, and (T-IIC)2 represents two-cell aggregates of the alveolar type II cells in the flow cytometry diagram.
B[a]P desorption contain a higher proportion of 9-OH-4,5-oxo-dGuo when compared to animals dosed with the UAP with medium and fast B[a]P release (Figure 2). In summary, both BNF pretreatment and UAP adsorption of B[a]P alters identity, levels, and persistence of B[a]P-DNA adducts in rat lungs.

**Alimentary Carcinogen Exposure and Enzyme Induction**

Urban air particulates deposited in the airways are relocated to the gastrointestinal tract through the mucociliary clearance process. Particulate-adsorbed carcinogens and enzyme inducers will follow the relocation pattern of the particulates. We studied, therefore, the effects of TCDD receptor ligand exposure on the small intestinal P450 enzyme activity with systemic distribution of tryptophan pyrolysate product, 3-amino-1,4-dimethyl-5H-pyridol-(4,3)-indole (TRP-P1), and covalent binding of B[a]P to liver DNA as end points. We detected an inducible level of P450IA1 in the proximal small intestine (57). Interestingly, increasing doses of BNF produced a 15- to 30-fold induction of the duodenal P450IA1 levels before any induction could be detected in the liver. At a BNF dose causing a 40-fold induction of the intestinal P450IA1, the liver enzyme level was induced only marginally with less than a 2-fold increase. Because UAP pass through the gastrointestinal tract relatively fast and because desorption of UAP-adsorbed compounds is a slow process, we believe that any substantial systemic P450 induction because of alimentary exposure of UAP is most unlikely.

The effects of alimentary BNF exposure was further studied in rats after per oral dosing of TRP-P1. This potent mutagen and carcinogen has been identified in the environment in UAP extracts at a concentration of 0.23 ± 0.17 pg/m³ (58). We therefore investigated the effect of BNF exposure on the autoradiographic distribution of orally dosed TRP-P1 in rats with a 40-fold induction of the small intestinal P450IA1 enzyme level. Following oral dosage of both inducer and [14C]TRP-P1, we observed an enhanced radioactivity retention in the small intestinal epithelium, the forestomach, and the esophagus. Interestingly, the lung also accumulated TRP-P1-derived [14C]-radioactivity, representing TRP-P1 or metabolites (manuscript in preparation).

**Enzyme Induction and 2-Nitrofluorene Metabolism in Lung Cells**

The lung metabolism of 2NF is induced by pretreatment of rats with BNF as observed in the isolated perfused and ventilated rat lung. The 2NF concentrations decline significantly faster in the perfusion buffer with lungs obtained from BNF-pretreated rats as compared to lungs from control animals (Figure 6A). When 2NF was administered by intratracheal administration, the most significant change was a decreased area under the 2NF concentration–time curve, proportional to an enhanced production of metabolites (Figure 6B) (37).

The effect of BNF pretreatment is not uniformly distributed throughout the lung because the lung cytochrome P450 enzymes are concentrated to specific cell categories (59,60). We also observed that rat lung
Clara cells and alveolar type II cells express different P450 enzymes with the consequence that the cells also metabolize 2NF with different capacity and regiospecificity. Pretreatment of rats with BNF-induced-P450IAl-associated aromatic hydroxylation of 2NF, which appears to be highly specific for the alveolar type II cells (manuscript in preparation).

In contrast to the inducible P450IAl enzyme, the constitutively expressed rat lung P450IIB1 enzyme is regulated in an age-dependent manner in the rat (61). The lung microsomal 2NF metabolism increased in parallel with the age-dependent up-regulation of lung P450IIB1. This increased capacity to form the mutagenic metabolite 9-OH-2NF changed steeply between three to four weeks after birth (61).

The lung-cell specific effects of BNF pretreatment was studied after isolation of lung cells by elutriation centrifugation. The cell categories studied were alveolar type II cells and Clara cells. The cell fractions that were used for metabolic studies or enzyme or mRNA analyses were analyzed by two-dimensional flow cytometry (2DFC) to characterize the homogeneity of the cells. The 2DFC analysis of two cell fractions are displayed in Figure 7. Figure 7A shows a representative analysis of dividing alveolar type II cells obtained from rat lungs by elutriation centrifugation. The solitary DNA peak, at the distance of 2 × n from the major peak, represents alveolar type II cells in division. B shows Clara cells. This cell fraction is contaminated with two-cell aggregates of alveolar type II cells. This contamination affects the 2NF metabolism profile of this cell fraction.

A high capacity for 9-hydroxylation of 2NF was detected in incubations with Clara cells (Figure 8A), which was in line with the observation of a Clara cell-specific P450IIB1 expression analyzed by mRNA Northern blot analysis (manuscript in preparation). Alveolar type II cells also possessed a considerable metabolic capacity but with preference for hydroxylation of 2NF on aromatic positions (X-ON-2NF). The total 2NF metabolism rate increased significantly in alveolar type II cells (Figure 8D) after pretreatment with BNF. A slight induction of X-hydroxylation also was seen, however, in Clara cell fractions (Figure 8C). This inducible X-hydroxylation that was detected in the Clara cell fraction probably is because of the presence of alveolar type II cell aggregates in this fraction, as observed in 2DFC analyses presented above. Significantly more X-OH-2NF and polyhydroxylated 2NF metabolites appeared in the HPLC chromatogram after incubation of alveolar type II cells obtained from BNF pretreated rats (manuscript in preparation). This result was not unexpected because cytochrome P450IAl is known to metabolize the structurally related carcinogen 2-acetylaminofluorene with a high preference for aromatic ring-hydroxylation (62,63). Pretreatment of rats with BNF also increased the P450IAl mRNA hybridization signal most extensively in Northern blot analyses of RNA extracts from type II cells (manuscript in preparation). This combination of a P450IAl enzyme induction in alveolar type II cells and the constitutive expression of P450IIB1 in the Clara cells affects the elimination of lung deposited 2NF as shown in the isolated perfused and ventilated lung (Figure 6B). The enhanced clearance observed after intratracheal dosage of 2NF to lungs from BNF pretreated rats probably is because of an induced level of alveolar type II cell P450IAl.

The metabolism of 2NF in human lung tissue was studied in incubations with 2NF added to a surgical resectate containing an adenocarcinoma and normal surrounding tissue.

Human lung tissue enzymes catalyze mainly 9-hydroxylation; although, a low degree of aromatic hydroxylation of 2NF also was detected. The 9-hydroxylation pathway that was catalyzed by human lung tissues was inhibited by addition of proadifen in analogy with our previous observations on the rat lung metabolism (manuscript in preparation). Similar enzymes generating similar

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**Table 1. In vitro 8-hydroxylation of deoxyguanosine residues in calf thymus DNA, catalyzed by urban air particulates.**

| Experimental system | Hydroxylation rate, 8-OH dG/10^9 dG/hr |
|---------------------|----------------------------------------|
| UAP-1 (4)           | 9.5 ± 1.6                              |
| UAP-2 (13)          | 3.2 ± 2.2                              |
| UAP-3 (5)           | 14.1 ± 2.5                              |
| UAP-4 (17)          | 11.7 ± 1.6                              |
| UAP-5 (1)           | 49.7 ± 1.3                              |
| Crocidolite asbestos| 11.5 ± 3.0                              |
| a + Catalase 100 μM | 0.6 ± 0.3                              |
| a + Catalase boiled | 7.1 ± 2.3                              |
| a + FeCl3, 3 μM     | 23.7 ± 1.9                              |
| a + FeCl3, 30 μM    | 36.2 ± 3.1                              |
| a + Desferal, 1 mM  | 1.4 ± 2.4                               |
| a + Desferal, 1 mM  | 3.2 ± 1.4                               |
| a + H2O2, 5 μg     | 38.1 ± 1.1                              |

Abbreviations: 8-OH dG, deoxyguanosine 8-hydroxylation; UAP, urban air particulates; FeCl3, ferric chloride; H2O2, hydrogen peroxide; HPLC, high-pressure liquid chromatography. Deoxyguanosine 8-hydroxylation in incubations with 1 mg DNA/ml was determined by HPLC. A control 8-hydroxylation rate was determined in control incubations to a level of 1.3 8-OH dG residues per 1 × 10^8 dG, which is subtracted from the data presented in Table 1. Data presented represents the mean value ± SD from five experiments. One incubation with 5 μg H2O2 was included as a positive control, causing deoxyguanosine 8-hydroxylation to change to a level of 22.4 ± 1.4 8-OH dG/10^9 dG.
2NF metabolites may be active thus both in man and the laboratory rat.

Urban Air Particulates and Oxygen Radicals

Reactive oxygen species are generated in aqueous solutions of urban air particulates, containing a complex mixture of polynuclear aromatic hydrocarbons and their oxidation products and derivatives together with mineral fibers, metal ions, and especially iron. The ability of different UAP samples to catalyze deoxyguanosine 8-hydroxylation (8-OH-dG) was therefore analyzed by HPLC chromatography, as shown in Figure 9 (51).

The UAP samples differed significantly in terms of catalytic activity (Table 1). The two most potent UAP samples did catalyze 8-OH-dG formation with a capacity comparable to tumor promoting asbestos. It is not known how reactive oxygen species were formed, if through generation of hydrogen peroxide, $\text{H}_2\text{O}_2$; superoxide anion, $\text{O}_2^-$; or hydroxyl radicals, $\cdot\text{OH}$. Superoxide anions and hydrogen peroxide have been identified in cigarette smoke and other fumes, however (64,65), and may originate from oxidation of aromatic polyphenols (31,32), which may originate from autooxidation of PAH. Hydrogen peroxide is of general lung-toxic interest because it also induces squamous metaplasia (66,67). Hydrogen peroxide is readily converted to the hydroxyl radical through the iron-catalyzed Fenton reaction: $\text{H}_2\text{O}_2 + \text{Fe}^{2+} \rightarrow \cdot\text{OH} + \text{OH}^- + \text{Fe}^{3+}$. Because iron is present together with the UAP samples, these theoretically should be expected to possess the capacity to catalyze hydroxyl radical formation. Inhibition of 8-OH-dG formation after addition of desferroxamine to the UAP incubations supports this hypothesis (Table 1).

Urban Air Particulates and Genotoxic Risk

The aim of this investigation was to study several levels of interaction whereby urban air particulates may influence metabolism availability and genotoxicity of chemical carcinogens. The mechanism(s) involved in UAP potentiation of carcinogenesis essentially is unknown still. We have shown that UAP adsorption of $\text{B}[\text{a}]\text{P}$ significantly alters several key events (i.e., bioavailability of the carcinogen and formation and persistence of specific DNA adducts). In animals dosed UAP-adsorbed $\text{B}[\text{a}]\text{P}$ characterized by slow carcinogen release, the lung DNA adduct levels increased continuously for eight weeks. We also observed that $\text{B}[\text{a}]\text{P}$ adsorption to UAP increased the DNA binding of $\text{B}[\text{a}]\text{P}$ by a factor of five. An increase, approximately 5-fold over prevalence, also has been reported in an epidemiological study of bronchogenic carcinoma among ferrous foundry workers (68,69). We therefore believe that the present observations support the hypothesis that UAPs may potentiate chemical carcinogen toxicity at several levels.

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