TOXICOLOGICAL SCIENCES **79,** 296–303 (2004)
DOI: 10.1093/toxsci/kfh122
Advance Access publication March 31, 2004

N-Acetylcysteine Prevents Lung Inflammation After Short-Term Inhalation Exposure to Concentrated Ambient Particles

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Received January 7, 2004; accepted February 26, 2004

Lung inflammation is a key response to increased levels of particulate air pollution (PM); however, the cellular mechanisms leading to this response are poorly understood. To determine whether oxidants are implicated in PM-dependent lung inflammation, we tested the ability of N-acetylcysteine (NAC) to prevent lung inflammation in a rat model of short-term exposure to concentrated ambient particles (CAPs). Adult Sprague-Dawley rats were exposed to either CAPs aerosols (CAPs mass concentration 1060 ± 300 $\mu g/m^3$) or filtered air (Sham controls) for 5 h. NAC-treated rats received 50 mg/kg (ip) NAC 1 h prior to exposure to CAPs. Oxidative stress and recruitment of inflammatory cells into bronchoalveolar lavage were evaluated 24 h after removal of the animals from the exposure chamber. Rats breathing CAPs aerosols showed significant oxidative stress, determined by the accumulation of thiobarbituric reactive substances (TBARS, 90 ± 15 pmol/mg protein; sham control: 50 ± 5 pmol/mg protein, $p < 0.02$) and oxidized proteins (1.6 ± 0.4 nmol/mg protein, sham: 0.70 ± 0.02 nmol/mg protein, $p < 0.01$) in their lungs. CAPs-induced oxidative stress was associated with increased numbers of polymorphonuclear leukocytes in bronchoalveolar lavage (BAL) (9 ± 2%; sham: 1.6 ± 0.5%, $p < 0.001$) and slight lung edema (wet/dry ratio: 4.77 ± 0.03, sham: 4.69 ± 0.02). No significant change was found in BAL protein concentration, total cell count, or lactate dehydrogenase (LDH) activity. NAC pretreatment effectively prevented CAPs-induced TBARS accumulation (30 ± 10 pmol/mg protein, $p < 0.006$), lung edema (4.64 ± 0.08, $p < 0.05$), and polymorphonuclear neutrophil (PMN) influx into the lungs (2.1 ± 0.5%, $p < 0.001$), but did not alter the protein carbonyl content. Histological evaluation of tissue samples confirmed the BAL findings. CAPs-exposed animals showed slight bronchiolar inflammation and thickened vessels at the bronchiole, whereas NAC treated animals showed no histological alterations. Regression analyses showed strong associations between increased TBARS accumulation and the CAPs content of Al, Si, and Fe, and trends of association between carbonyl content and Cr and Na concentrations, and between BAL PMN count and Cr, Zn, and Na. These data demonstrate that oxidants are critical mediators of the inflammatory response elicited by PM inhalation.

Key Words: **reactive oxygen species; oxidative stress; particulate air pollution; inflammation; CAPs.**

Ambient air particles are complex mixtures of organic and inorganic components that may vary in proportion and composition daily. Epidemiological studies show that transient high-level increases in ambient particulate matter (PM) of respirable size are strongly associated with increased exacerbation of respiratory disease, cardiopulmonary morbidity, and mortality (reviewed in Schwela, 2000). The respiratory effects include exacerbation of preexisting diseases, such as asthma and obstructive pulmonary disease, and increased hospital admissions for pneumonia.

Proinflammatory and toxic effects of PM have been observed in the laboratory, in human subjects, in animal models, and in cells in culture. PM inhalation or intratracheal instillation was shown to invoke an inflammatory response in humans (Ghio and Devlin, 2001; Ghio *et al.*, 2001), dogs (Clarke *et al.*, 2000b; Godleski *et al.*, 2000), and rats (Clarke *et al.*, 1999, 2000a; Hatch *et al.*, 1985; Kennedy *et al.*, 1998; Li *et al.*, 1997; Saldiva *et al.*, 2002). Critical components of the inflammatory response to PM are the release of macrophage- and pneumocyte-derived cytokines (Kennedy *et al.*, 1998; Li *et al.*, 1997) and the concomitant recruitment of neutrophils (Ferin *et al.*, 1992; Kennedy *et al.*, 1998; Li *et al.*, 1996, 1997).

Increased production of reactive oxygen species (ROS) by PM is suggested by the finding that many of the proinflammatory genes induced upon *in vivo* exposure to PM (TNF-α, and β, TGF-β, γ-IF, IL-6, and IL-8 among others, Shukla *et al.*, 2000) are regulated by redox-sensitive transcription factors such as NF-κB, AP-1 and C/EBP. Activation of some of these transcription factors and increased transcription of downstream genes have been also demonstrated *in vitro* in alveolar and bronchial epithelial cell lines treated with PM (Jimenez *et al.*, 2000; Kennedy *et al.*, 1998; Shukla *et al.*, 2000). A role for ROS in these systems is further supported by the prevention of cytokine upregulation by enzymatic (SOD and catalase) and

Data presented in part at the Annual Meeting of the American Thoracic Society 2003, Seattle, Washington.

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nonenzymatic (N-acetylcysteine, NAC) antioxidants (Jimenez *et al.*, 2000; Kennedy *et al.*, 1998; Shukla *et al.*, 2000).

We have previously shown that inhalation exposure to concentrated ambient particles (CAPs), but not inert particles, leads to time-dependent increases in the steady-state concentrations of oxidants in the lung and heart (Gurgueira *et al.*, 2002). The oxidative stress imposed by CAPs was associated with the metal content of the particles in a tissue specific manner, and led to mild increases in lung and heart edema. In this study, we investigated the role of ROS in the development of CAPs-induced pulmonary inflammation and toxicity. Our data show that short-term inhalation exposure to CAPs leads to significant accumulation of oxidized lipids and proteins in the lung. Pulmonary oxidative stress was associated with increased polymorphonuclear neutrophil (PMN) count in bronchoalveolar lavage (BAL), PMN infiltration. These biological effects were prevented and pretreatment of the animals with NAC at a dose that prevented accumulation of oxidants.

MATERIALS AND METHODS

Concentrated ambient particles (CAPs). The Harvard Ambient Particle Concentrator (HAPC) concentrates ambient air particles for subsequent aerosol exposure of animals (Sioutas *et al.*, 1995). The principle of virtual impaction is used to concentrate ambient particles in the size range of 0.1–2.5 μm (fine particles, concentration factor: 26 $\pm$ 4, [Sioutas *et al.*, 1995]). CAPs remain in suspension without physical or chemical alteration for inhalation exposures or for collection onto filters for mass and composition analysis. Mass concentrations and the size of the particles, determined gravimetrically and using a microorifice impactor, respectively, were monitored continuously during the operation of the HAPC. Trace metal concentrations were determined using x-ray fluorescence (Chester LabNet, Tigrad, OR). The CAPs total mass and elemental composition of the CAPs used in this study are presented in Table 1. The average CAPs composition was comparable to the values obtained previously (Gurgueira *et al.*, 2002). Twelve out of the eighteen metals analyzed were present at equivalent concentrations. However, in average, these samples had three-fold higher concentrations of Al, Zn, and Br, five-fold higher concentrations of S and Mn, a four-fold higher total mass concentration, and almost no Cl.

Exposure to CAPs. Pathogen-free male Sprague Dawley rats (Taconic Farms, Germantown, NY) weighing 250–300 g were used. Animals were fed a conventional laboratory diet and water *ad libitum.* Rats were exposed to CAPs aerosols (CAPs group) or filtered air (control group) as described previously (Clarke *et al.*, 2000a). The animals were awake and unrestricted during the exposures. The CAPs and control groups were exposed and tested simultaneously. The exposure was repeated ten times on ten different days from June 20 to August 16, 2002 (average CAPs mass concentration: 1228 $\mu g/m^3$, Table 1). Each exposure was carried out for 5 h and included animals exposed to CAPs or filtered air (sham controls) with and without N-acetylcysteine pretreatment. Four animals (1 per group: CAPs, CAPs/NAC, sham, and sham/NAC) were assessed, 24 h after exposure, for oxidative stress and tissue damage, and eight animals (two per group) were assessed for markers of inflammation. On July 31, August 9, 14, and 16, additional animals (two per group each day) were added to the exposure protocol to be processed for histopathology. The temperature in the room and chamber was 25°C.

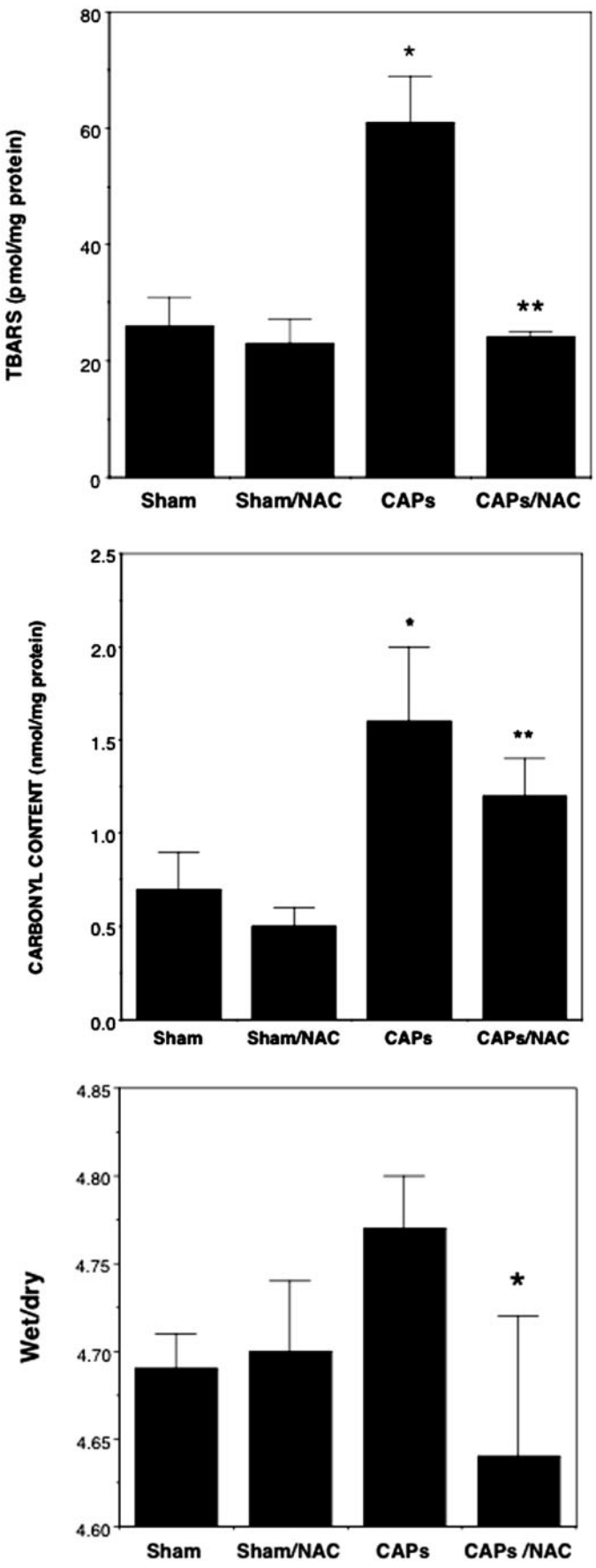
Tissue preparation. At the end of the exposure, animals were removed from the HAPC and kept in room air for 24 h. The lungs were then removed and frozen in a dry ice bath. Separate samples were taken for the determination of thiobarbituric acid-reactive substances (TBARS), carbonyl content, and water content. Samples to be processed for the determination of TBARS and carbonyl content were homogenized in 120 mM KCl, 30 mM phosphate buffer (pH 7.2), added with protein inhibitors (1 μg/ml leupeptin, 1 μg/ml aprotinin, 10 μg/ml soybean trypsin inhibitor, 1 μg/ml pepstatin, and 0.5 mM PMSF) at 0–4°C. The suspensions were centrifuged at 600 $\times$ *g* for 10 min at 0–4°C to remove nuclei and cell debris. The pellets were discarded and the supernatants were used as homogenates.

Determination of TBARS. For measurements of TBARS, homogenates were precipitated with 10% trichloroacetic acid (TCA), centrifuged, and incubated with thibarbituric acid (Sigma, Chem. Co.) for 1 h at 100°C. TBARS were extracted using butanol (1:1). After centrifugation, the fluorescence of the butanol layer was measured at 515nm excitation and 555 nm emission using a PTI spectrofluorometer (Photon Technology International, Lawrenceville, NJ). The amount of TBARS formed was expressed in picomoles per milligram of protein. Malondialdehyde standards were prepared from 1,1,3,3,-tetramethoxypropane (Esterbauer and Cheeseman, 1990). Protein concentration in homogenates was measured by Lowry method (Lowry *et al.*, 1951) using

TABLE 1
Elemental analysis of CAPs

Date	CAPs mass	Na	Mg	Al	Si	S	Cl	K	Ca	Ti	V	Cr	Mn	Fe	Ni	Cu	Zn	Br	Cd	Ba	Pb
6/20/02	540	0.61	1.04	4.90	14.6	33.81	0.39	3.42	6.97	0.59	0.12	0.05	0.34	12.49	0.11	0.24	1.51	0.16	0.00	0.86	0.11
6/27/02	2338	0.00	2.70	4.77	11.8	348.3	0.00	4.20	4.00	0.79	0.10	0.00	0.86	10.67	0.08	0.19	2.19	0.35	0.08	1.42	0.17
7/9/02	1196	0.00	5.52	5.96	13.1	23.53	0.34	9.50	5.81	1.23	0.08	0.00	0.93	14.58	0.05	0.36	1.44	0.15	0.01	1.93	0.16
7/11/02	485	0.00	2.82	2.85	8.71	6.83	0.35	3.35	5.17	0.64	0.04	0.00	1.15	11.07	0.00	0.10	1.75	0.05	0.00	0.88	0.00
7/16/02	1330	4.35	1.11	4.58	11.4	167.0	0.00	3.93	2.62	0.54	0.08	0.01	0.59	10.78	0.02	0.18	1.54	0.22	0.00	0.38	0.12
7/24/02	2159	7.84	0.38	3.22	8.03	287.1	0.10	3.76	2.50	0.51	0.12	0.02	0.49	6.91	0.08	0.13	2.23	0.40	0.00	0.51	0.11
7/31/02	706	0.76	1.44	2.84	6.52	88.05	0.09	1.95	2.78	0.33	0.03	0.03	0.41	6.16	0.03	0.22	0.56	0.12	0.02	0.18	0.07
8/9/02	150	0.00	1.19	3.13	7.06	3.12	0.39	1.40	3.35	0.41	0.02	0.00	0.81	8.43	0.00	0.06	1.36	0.01	0.00	0.68	0.01
8/14/02	2520	1.96	1.18	13.1	40.3	352.6	0.00	7.22	7.70	1.11	0.12	0.06	0.81	18.54	0.08	0.26	1.94	0.38	0.00	0.94	0.22
8/16/02	858	9.84	1.93	6.81	18.7	109.0	0.17	4.45	5.01	0.55	0.08	0.01	0.48	9.48	0.03	0.10	1.30	0.18	0.00	0.57	0.05
Average	1228	2.54	1.93	5.21	14.03	141.9	0.18	4.32	4.59	0.67	0.08	0.02	0.69	10.91	0.05	0.18	1.58	0.20	0.01	0.83	0.10
Minimum	150	0.00	0.38	2.85	7.06	3.1	0.00	1.40	2.50	0.41	0.02	0.00	0.34	6.16	0.00	0.06	0.56	0.01	0.00	0.18	0.00
Maximum	2520	7.84	5.52	13.10	40.30	348.3	0.39	9.50	7.70	1.23	0.12	0.06	1.15	18.54	0.11	0.36	2.23	0.38	0.08	1.93	0.22

Note: All values are in $\mu g/m^3$.



bovine serum albumin as standard. Measurements were carried out in a Perkin Elmer Lambda 40 spectrophotometer.

Determination of carbonyl content. The content of carbonyl groups in oxidatively modified proteins was measured in lung homogenates by determining the amount of 2,4-dinitrophenylhydrazone formed upon reaction with 2,4-dinitrophenylhydrazine (Fields and Dixon, 1971). After precipitation of nucleic acids with 1% streptomycin sulfate, samples (>1mg of protein per ml) were treated with 2 mM 2,4-dinitrophenylhydrazine at room temperature, usually for 1 h. Proteins were precipitated with 10% TCA, washed with ethanol/ethyl acetate (1:1), and redissolved in 6 M guanidine hydrochloride–20 mM potassium phosphate (pH 2.3) (Levine *et al.*, 1994). Carbonyl content was calculated from the absorbance maximum of 2,4-dinitrophenylhydrazone at 390 nm normalized to the absorbance at 350 nm, with an $\varepsilon_{390-350}$ of 22 mM^{-1} cm^{-1} (Levine *et al.*, 1994). Results are expressed in nanomoles of carbonyl groups per milligram of protein.

Bronchoalveolar lavage. Rats exposed to CAPs or filtered air were anesthetized with sodium pentobarbital (50mg/kg body weight) 24 h after exposure, and their lungs lavaged through the trachea using 5-ml aliquots of PBS (total volume, 50 ml). Each aliquot represent one in and out recovery of fluid. The recovered fluid was centrifuged (400g) at 4°C, and the supernatant from the first lavage was saved for measurement of protein level and lactate dehydrogenase (LDH) activity. Total cell counts were determined after trypan blue stain using a Newbauer chamber. Differential cell counts were performed using modified Wright-Giemsa stain in cytospin preparations (200 cells counted per sample). Total protein levels, as measure of vascular permeability, and LDH activity, as indicator of general toxicity, were measured in the supernatant of the first lavage using the method of Lowry *et al.* (Lowry *et al.*, 1951) and standard kits (Sigma Chem. Co.), respectively. These measurements were carried out in a Perkin Elmer Lambda 40 spectrophotometer.

Lung edema. Lung samples (~100 mg), taken from the same animals used for the determinations of TBARS and carbonyl content samples were weighed and then dried in a convention oven (~80°C) and reweighed 24 h after to obtain the wet/dry ratios.

Histopathology. The final pair of rats was euthanized using an overdose of sodium pentobarbital 24 h after exposure. The lungs were excised and fixed by intratracheal instillation of 2.5% glutaraldehyde in 0.1 M potassium phosphate buffer at constant pressure of 20 cm H_2O. After fixation, all lung lobes (except for the cardiac lobe) were cut horizontally into uniform 2-mm sections with a guided razor blade. Each section was numbered, and one randomly selected section from each lobe was processed for histology. Tissue samples were embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin for analysis by light microscopy. Histologic slides were coded for blinded observation, and the observer was unaware of the code until the analysis was completed.

Statistics. The numbers in tables and the bars in figures indicate the mean value ± standard error of the mean (SEM) of 4–10 independent experiments. Data were analyzed statistically by factorial analysis of variance (ANOVA) followed by Fisher's test for comparison of the means. For elemental composition correlation analyses, separate linear regression models were fit using actual elemental concentration univariately as predictors. All statistical analyses were performed using Statview software for Macintosh.

Animal Care. The Harvard School of Public Health is accredited by the American Association for the Accreditation of Laboratory Animal Care, meets

FIG. 1. Oxidant damage in CAPs-exposed rats. Rats were exposed to CAPs (average mass concentration: 1060 ± 300 $\mu g/m^3$) or filtered air (sham controls) for 5 h. At the end of the exposure the lungs were removed, processed, and assayed for TBARS (**A**) and protein carbonyl content (**B**) as described in Materials and Methods. The bars represent the mean of ten determinations ± SEM. *$p < 0.0005$ in (A) and $p < 0.01$ in (B) compared to the respective sham controls. **$p < 0.0002$ in (A) and $p < 0.002$ in (B) compared to CAPs.

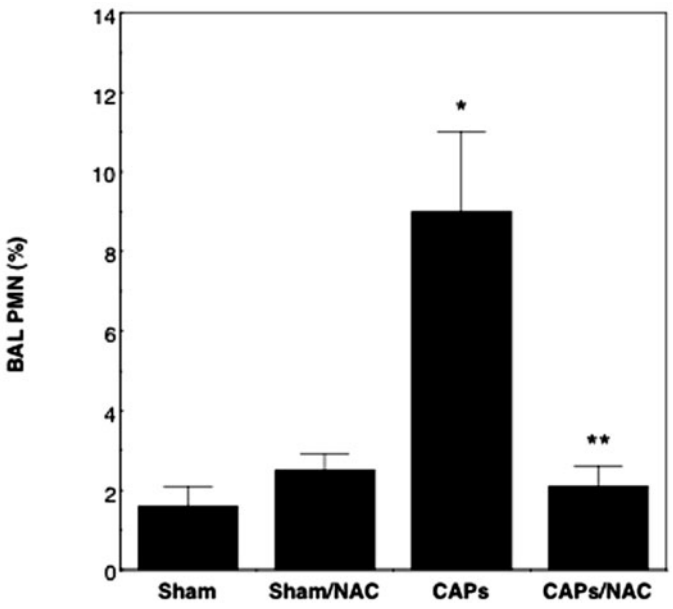


FIG. 2. PMN influx in CAPs-exposed rats. BAL samples were collected 24 h after the end of the exposure to CAPs. Total and differential cell counts were performed as described in Materials and Methods. The bars represent the mean of ten determinations ± SEM. *$p < 0.0004$ compared to the respective sham controls; **$p < 0.001$ compared with CAPs exposure.

National Institutes of Health standard as set forth in the "Guide for the Care and Use of Laboratory Animals," and accepts as mandatory the NIH "Principles for the Use of Animals." The principal investigator and laboratory personnel involved in this project have demonstrated competence in the care, use, and handling of laboratory animals. We give assurance of humane practice in animal maintenance and experimentation. All protocols of exposure and other procedures used in this study have been approved by the Harvard Animal Use Committee.

RESULTS

Oxidative Stress and Oxidative Damage in Rats Exposed to CAPs Aerosols

In agreement with our previous findings, inhalation exposure to CAPs led to significant increases in the accumulation of oxidized lipids (Fig. 1A) and proteins (Fig. 1B). Rats breathing CAPs for 5 h showed greater than two-fold increases in their pulmonary levels of TBARS (Fig. 1A) and protein carbonyls (Fig. 1B). Pretreatment of the animals with 50 mg/kg NAC, 1 h prior to the initiation of the exposure effectively prevented the CAPs-dependent increases in oxidized lipids (Fig. 1A) and partially prevented the accumulation of oxidized proteins (Fig. 1B).

Oxidant-Dependent Inflammation and Toxicity after CAPs Inhalation

To test the hypothesis that PM-induced pulmonary inflammation and toxicity are mediated by ROS, we evaluated the effect of NAC pretreatment on a series of markers of inflammation and damage in rats exposed to CAPs. Bronchoalveolar lavage fluid was collected from animals exposed to CAPs aerosols or filtered air for 5 h and evaluated for total and differential cell count, LDH activity, and total protein levels as described in the methods section. Inhalation exposure to CAPs led to significant accumulation of PMN leukocytes in BAL (Fig. 2 and Table 2). PMN influx was not accompanied by significant changes in LDH activity and total protein levels (Table 3). CAPs-dependent lung inflammation was effectively prevented by preadministration of NAC (Fig. 2 and Table 2). Histological evaluation of tissue samples from animals treated with CAPs or filtered air in the presence or absence of NAC confirmed the BAL findings (Fig. 3). CAPs-exposed animals showed slight bronchiolar inflammation and thickened vessels at the bronchiole (Fig. 3B) as previously reported (Batalha *et al.*, 2002; Saldiva *et al.*, 2002). However, CAPs-exposed animals pretreated with NAC showed no inflammation in the bronchiolar location and thin-walled vessels (Fig. 3C).

Finally, NAC treatment significantly decreased the wet/dry ratio, a measurement of global tissue damage, in CAPs exposed animals (Fig. 4).

TABLE 2
Differential Cell Count in BAL Fluid from Rats Exposed to CAPs

		Macrophages		PMN		Lymphocytes		Eosinophils	
	Total cells	10^{-6}	(%)	$\times 10^{-4}$	(%)	$\times 10^{-4}$	(%)	$\times 10^{-2}$	(%)
Sham	2.7 ± 0.5	2.6 ± 0.5	97 ± 1	4 ± 1	1.6 ± 0.5	3 ± 1	1.2 ± 0.2	2.6 ± 2.6	0.1 ± 0.1
CAPs	2.4 ± 0.3	2.2 ± 0.2	90 ± 2[a]	21 ± 4	9 ± 2[a]	1.7 ± 0.5	0.7 ± 0.2	1.6 ± 1.6	0.07 ± 0.07
Sham/NAC	1.6 ± 0.3	1.5 ± 0.3	96 ± 1[b]	4 ± 1	2.5 ± 0.4[b]	2.0 ± 0.5	1.3 ± 0.4	0	0
CAPs/NAC	1.7 ± 0.3	2.0 ± 0.3	97 ± 1[b]	3.4 ± 0.7	2.1 ± 0.5[b]	1.9 ± 0.7	0.8 ± 0.2	0	0

Note: $n = 10$.
[a] $p < 0.001$ versus sham.
[b] $p < 0.001$ versus CAPs.

TABLE 3
LDH Activity and Total Protein in BAL Fluid from Rats Exposed to CAPs

	Total protein (mg/ml)	LDH (Units/ml)
Sham	0.7 ± 0.1	110 ± 20
CAPs	0.8 ± 0.1	160 ± 20
Sham/NAC	0.6 ± 0.1	130 ± 20
CAPs/NAC	0.9 ± 0.3	170 ± 40

Note: $n = 8$, $p > 0.05$

Metal Content as a Determinant of CAPs Pulmonary Effects

One of the advantages of working with CAPs aerosols is that they reflect the day-to-day changes in composition typical of urban environments, and in that way they provide a set of samples with a wide range of metal concentrations suitable for statistical analyses (Table 1). Using univariate regression we identified several components with significant associations to increased TBARS accumulation in the lung (Table 4). Due to the strong effect of CAPs inhalation on this outcome, many elements show positive correlations to the TBARS levels. However, the stronger and more significant associations were found for Al, Si, and Fe (Table 4). We failed to see significant associations between metal concentrations and lung carbonyl content or PMN influx into the lung, probably due to the somewhat limited number of days analyzed. However, there was a trend of association between carbonyl content and Cr and Na concentrations, and between BAL PMN count and Cr, Zn, and Na (Table 4).

DISCUSSION

The ability of PM to increase the intracellular production of ROS, although assumed to be essential for their biological effects, has not been extensively tested *in vivo*. In a recent report we showed that inhalation exposure to CAPs, following the same protocol used in this paper, increases by two-fold the steady-sate concentration of ROS in the rat lung and heart (Gurgueira *et al.*, 2002). Our current data show that CAPs-dependent increases in ROS are sufficient to cause accumulation of oxidized lipids and proteins (Fig. 1). CAPs exposure led to about a two-fold increase in the levels of TBARS and protein carbonyls, the same magnitude of increase previously observed for ROS concentrations (Gurgueira *et al.*, 2002). Pretreatment of the animals with 50 mg/kg NAC (ip) effectively prevented accumulation of oxidants as shown by TBARS (Fig. 1A). On the other hand, NAC treatment could only partially prevent accumulation of oxidized proteins in CAPs-exposed animals (Fig. 1B). This relative inefficiency could reflect constant accumulation of oxidized proteins over the 5 h of exposure and consumption of NAC.

Our data suggest that CAPs-dependent oxidative stress is associated with the development of lung inflammation. The ability of CAPs from different urban areas to promote lung inflammation has been previously reported. Inhalation exposure to CAPs from the New York area for 6 h was shown to increased the mRNA levels of proinflammatory cytokines (including IL-6, TNF-α, and IFN-γ) in the mice lung 24 h after exposure (mass concentration: 300 $\mu g/m^3$) (Shukla *et al.*, 2000). Similarly, 3-day exposures to CAPs aerosols from the Boston area (5 h/day, mass concentration: 205–733 $\mu g/m^3$) were shown to decrease BAL macrophages and increase BAL lymphocytes, PMNs, and total protein in rat 24 h after exposure (Clarke *et al.*, 1999; Saldiva *et al.*, 2002). However, composition and timing seem to be essential variables in the development of the proinflammatory effects of CAPs, since similar protocols of exposure run in the Research Triangle Park area in North Carolina (3 days, 6 h/day, mass concentration: 265–1200 $\mu g/m^3$) did not elicit inflammatory responses when measured immediately after exposure (Kodavanti *et al.*, 2000).

In this study we found strong associations between the accumulation of oxidized lipids (TBARS) and the CAPs content of Al, Si, Fe, Cu, Pb, and K (Table 4). However, due to the

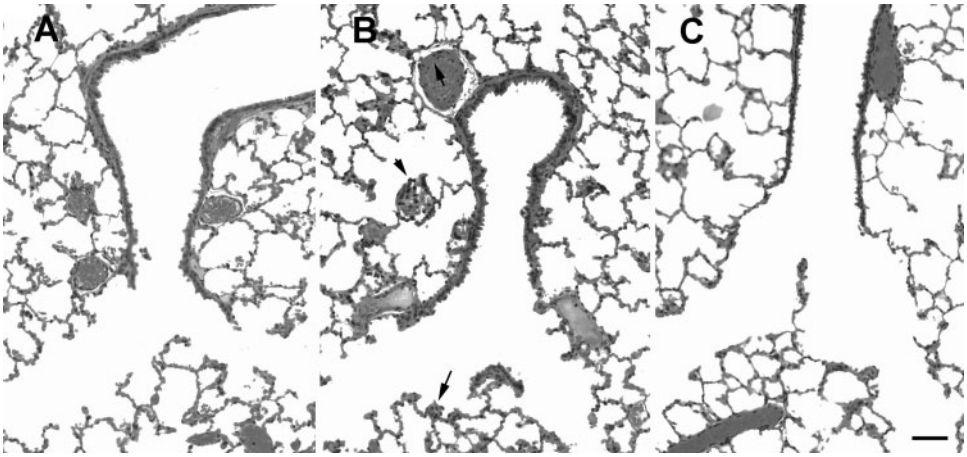


FIG. 3. Morphological appearance of lung tissue in selected areas illustrating the responses of control and NAC-treated rats to CAPs exposure. (A) In sham rats, the bronchoalveolar area has normal histology. (B) In CAPs-exposed rats, thickened vessels walls (arrow in vessel) and slight inflammation in the area of the bronchiolar alveolar junction are visible (see arrows in lung). (C) In CAPs-exposed rats pretreated with NAC, lung histology was normal. Scale bar = 50 μm.

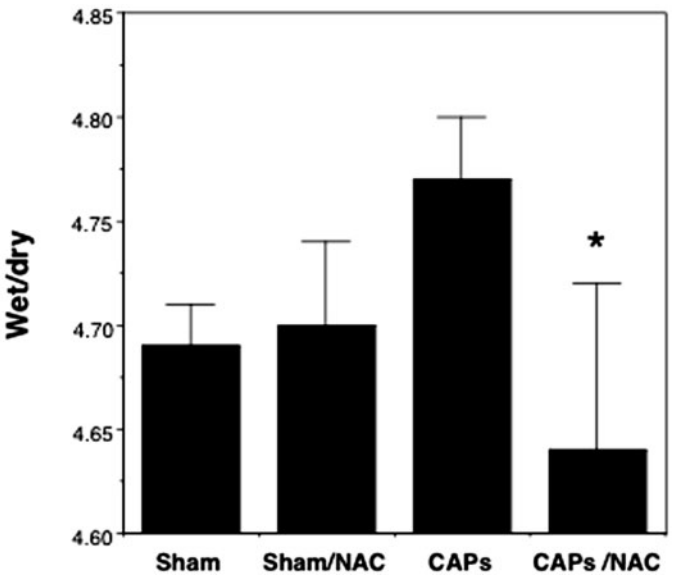


FIG. 4. Lung edema in CAPs-exposed rats. Animals were exposed to CAPs as described in Fig. 1. Lung samples were collected simultaneously for the determination of the wet/dry ratios. The bars represent the mean of four determinations ± SEM. *$p < 0.05$ compared to CAPs exposure.

limited size of the dataset and the less dramatic effect of CAPs inhalation on the lung carbonyl content and BAL PMN count, we could only see trends of association for these outcomes. Nonetheless, the suggested association between BAL PMN count and Zn is in agreement with previous data from the Godleski's group in a model of exposure to CAPs, for 6 h a day on three consecutive days (Clarke *et al.*, 2000b; Saldiva *et al.*, 2002).

Also in agreement with previously reported data, we found that CAPs-exposed animals displayed bronchiolar inflammation (Saldiva *et al.*, 2002) and thickening of blood vessels (Batalha *et al.*, 2002) (Fig. 3). These observations, combined with the increase in lung edema reported in a previous paper (Gurgueira *et al.*, 2002), indicate mild but significant toxicity by CAPs.

In vitro data show that, in some cases, the toxicity induced by PM emission components and surrogates can be ameliorated by antioxidants. Residual oil fly ash-induced mucin secretion and cytotoxicity in airway epithelial cells were attenuated by preadministration of dimethylthiourea (Jiang *et al.*, 2002). NAC pretreatment was shown to significantly prevent TNF-α production in primary alveolar macrophages treated with ultrafine nickel particles (Dick *et al.*, 2003) and to protect a macrophage cell line (THP-1) against diesel exhaust particle chemicals (Li *et al.*, 2002). However, under similar experimental conditions, bronchial epithelial cells were not protected by NAC against diesel emission particles toxicity (Li *et al.*, 2002).

TABLE 4
Statistical Parameters for Lung TBARS, Carbonyl Content, and PMN Count, and CAPs Elemental Components

	Lung TBARS		Lung carbonyls		PMN	
Element	r^2	p	r^2	p	r^2	p
Na	0.098	0.237	0.363	0.114	0.152	0.109
Mg	0.148	0.141	0.147	0.349	0.008	0.723
Al	0.445*	0.005*	0.045	0.626	0.079	0.259
Si	0.422*	0.007*	0.082	0.493	0.081	0.253
S	0.033	0.501	0.014	0.783	0.047	0.387
Cl	0.008	0.741	0.083	0.490	0	0.941
K	0.357*	0.015*	0.117	0.408	0.016	0.613
Ca	0.162	0.122	0.138	0.366	0.014	0.637
Ti	0.283	0.034	0.141	0.360	0.015	0.634
V	0.231	0.059	0.071	0.522	0.051	0.186
Cr	0.003	0.834	0.363	0.114	0.140	0.126
Mn	0.137	0.158	0.213	0.250	0.039	0.432
Fe	0.385*	0.010*	0.132	0.376	0.097	0.208
Ni	0.038	0.469	0.003	0.906	0.003	0.832
Cu	0.341*	0.018*	0.009	0.826	0.039	0.433
Zn	0.138	0.157	0.197	0.271	0.113	0.173
Br	0.104	0.223	0.016	0.766	0.081	0.252
Cd	0.005	0.796	0.012	0.797	0.054	0.352
Ba	0.122	0.185	0.194	0.274	0.010	0.700
Pb	0.350*	0.016*	0.001	0.934	0.043	0.408
Total mass	0.173	0.179	0.162	0.502	0.048	0.452

*The most significant associations are indicated with an asterisk.

Due to its potential for clinical use, NAC has been extensively tested as a generic antioxidant in *in vivo* models of oxidant-mediated toxicity. NAC has been successfully used to prevent PMN influx and lung damage in models of exposure to cigarette smoke (Balansky *et al.*, 1992), paraquat intoxication (Hoffer *et al.*, 1993), and carrageenan-induced pleurisy (Cuzzocrea *et al.*, 2001). The mechanism by which NAC prevents inflammation would include inhibition of ROS production in response to stimuli, with concomitant decreases in NFκB activation and expression of cytokine-induced neutrophil chemoattractant (Blackwell *et al.*, 1996). In models of preexisting inflammation, it has been shown that NAC can also modulate phagocytotic activity by suppressing PMN oxidative burst (Koch *et al.*, 1996; Stolarek *et al.*, 2002; Villagrasa *et al.*, 1997), and by potentiating host defense (Koch *et al.*, 1996; Villa *et al.*, 2002). On the other hand, treatment with NAC failed to prevent TNF-α-mediated MCP-1 up-regulation in vascular smooth muscle cells (De Keulenaer *et al.*, 2000), only reduced LPS-induced neutrophil infiltration at high doses (500 mg/kg) (Rockse *et al.*, 2000), and even transiently increased tissue damage and oxidative stress in humans subjected to acute muscle injury (Childs *et al.*, 2001).

Here, we applied an *in vivo* model of inhalation exposure to "real world" particles to demonstrate the central role of ROS in PM biological effects. Our data show that NAC, at a dose sufficient to prevent increase in ROS and accumulation of TBARS and to partially reduce protein oxidation, effectively prevented CAPs-induced inflammation. The observed preventive effect of NAC suggests that treatment with low doses of this antioxidant could be used to ameliorate the toxic effects of particulate air pollution.

ACKNOWLEDGMENTS

We thank Mr. Gonzalo Bedia-Diaz for technical assistance. C.R.R. acknowledges funding from the FFFCMPA (Brazil). This work was supported by grants from NIH (RO1 HL/ES68073 and P01 ES08129), and a Research Award from the U.S. EPA (R827353).

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