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OXIDATIVE INJURY IN THE LUNGS OF NEONATAL RATS FOLLOWING SHORT-TERM EXPOSURE TO ULTRAFINE IRON AND SOOT PARTICLES

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Greater risk of adverse effects from particulate matter (PM) has been noted in susceptible subpopulations, such as children. However, the physicochemical components responsible for these biological effects are not understood. As critical constituents of PM, transition metals were postulated to be involved in a number of pathological processes of the respiratory system through free radical-medicated damage. The purpose of this study was to examine whether oxidative injury in the lungs of neonatal rats could be induced by repeated short-term exposure to iron (Fe) and soot particles. Sprague Dawley rats 10 d of age were exposed by inhalation to two different concentrations of ultrafine iron particles (30 or 100 μg/m^3) in combination with soot particles adjusted to maintain a total particle concentration of 250 μg/m^3. Exposure at 10 d and again at 23 d of age was for 6 h/d for 3 d. Oxidative stress was observed at both Fe concentrations in the form of significant elevations in glutathione disulfide (GSSG) and GSSG/glutathione (GSH) ratio and a reduction in ferric/reducing antioxidant power in bronchoalveolar lavage. A significant decrease in cell viability associated with significant increases in lactate dehydrogenase (LDH) activity, interleukin-1-beta (IL-1β), and ferritin expression was noted following exposure to particles containing the highest Fe concentration. Iron from these particles was shown to be bioavailable in an in vitro assay using the physiologically relevant chelator, citrate. Data indicate that combined Fe and soot particle exposure induces oxidative injury, cytotoxicity and pro-inflammatory responses in the lungs of neonatal rats.

As critical constituents of particulate matter (PM), transition metals were postulated to be involved in a number of pathological and physiological processes of the respiratory system through free radical-medicated damage (Valavanidis et al., 2005; Risom et al., 2005; Sorensen et al., 2005; Zhou et al., 2003). Humans are commonly exposed to iron (Fe) and soot particles from a variety of emission sources of PM. Iron was found to be the prevalent catalytic metal in all size ranges of the ambient PM present in Los Angeles (Hughes et al., 1998), and the highest concentration of Fe in ultrafine particles was found to be in the size range of 0.056–0.1 μm in 7 Southern California cities (Cass et al., 2000).

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Potential sources for transition metals in the atmosphere are multifarious. Larger particles (i.e., >2.5 μm) containing metals may be derived from crustal dusts, while ultrafine particles are more likely to originate from high-temperature combustion sources. Iron is a well-known soot suppressant that might be emitted into the atmosphere in the form of ultrafine particles. For example, ferrocene has been commonly used as fuel additive for soot suppression. Iron oxide generated from the decomposition and oxidation of ferrocene is supersaturated and rapidly nucleates into numerous nano-sized particles that serve as sites for carbon deposition. Concentric rings of carbon laid down on Fe particles form an Fe and soot particle matrix (Boncyzk, 1991; Ritrievi et al., 1987). Soot is primarily composed of elemental carbon and also contains limited amounts of oxygen, nitrogen, hydrogen, and polyaromatic hydrocarbons (PAH). The emission sources of soot include diesel engines, fossil fuels, and wood smoke. Soot is thermodynamically involved in the reduction of Fe oxide in the flame at high temperatures (Boncyzk, 1991; Ritrievi et al., 1987; Zhang & Megaridis, 1996) and is typically considered to exert little effect when inhaled alone (Jakab, 1992, 1993; Jakab & Hemenway, 1994). Since ultrafine particles of soot have large surface areas and mass ratio, soot might also act as a carrier for copollutants, such as transition metals, and might transport co-pollutants into the respiratory tract. Formation of these complex particles may influence deposition and clearance from the lungs, thus, changing biological potential (Lindenschmidt & Witschi, 1990; Oberdorster, 2001; Schlesinger, 1995; Sun et al., 1989).

Although the major focus of studies relating ambient PM and adverse health effects has been in adults, a number of epidemiological studies showed that air pollution is also associated with respiratory morbidity, mortality, and decrements in pulmonary function and growth in children (Delfino et al., 2008; Tecer et al., 2008; Salvi, 2007; Gauderman et al., 2002; Calderon-Garciduenas et al., 2000; Conceicao et al., 2001; Horak et al., 2002; Jedrychowski et al., 1999; Zhang et al., 2002). The response of a child to particles may be entirely different from that of an adult, based on differences in ventilatory rates or maturation of metabolic, immune, neural, and anatomical systems (Foos et al., 2008). Furthermore, cellular differentiation, proliferation, physiological function, and xenobiotic-metabolizing enzymes within the respiratory system rapidly change during postnatal growth. Exposure of the respiratory tract to environmental toxicants during this time has the potential to significantly affect the maturation, growth, and function of critical elements compromising this system (Pinkerton & Joad, 2000). However, little is known regarding the susceptibility and environmental characteristics that may place children at greater risk from exposure to PM. A study that is designed to address the association between adverse effects and specific chemical composition could provide meaningful information to better understand the health effects of PM on children during development. Therefore, the purpose of this study was to examine the responses of inhaled Fe and soot particles generated by a diffusion flame system in the respiratory system of rapidly growing neonatal rats. To do this, Sprague-Dawley rats 10 d and 23 d of age were exposed by inhalation to 2 different levels of ultrafine Fe particles (30 $\mu g/m^3$ and 100 $\mu g/m^3$) in combination with soot particles adjusted to maintain a total particle concentration of 250 $\mu g/m^3$.

MATERIALS AND METHODS

Chemicals

Acetylene and ethylene were purchased from Airgas (Sacramento, CA). Iron pentacarbonyl, reduced glutathione, glutathione disulfide (GSSG), glutathione reductase, 5,5′-dithiobis(2-nitrobenzoic) acid (DTNB), NADPH, 2-vinylpyridine, ferrous sulfate, ferric chloride, tripyridytriazine (TPTZ), citrate, and ferrozine were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO).

Iron and Soot Particle Generation System

A diffusion flame system was used to generate an aerosol of soot and Fe oxide as described

previously (Yang et al., 2001). Briefly, the fuel was a mixture of acetylene and ethylene. Concentrations of Fe oxide and soot particles in the post flame gases were controlled independently. Iron was introduced by passing ethylene over liquid Fe pentacarbonyl. The aerosol emission from the flame was diluted by secondary air to control the actual particle concentration to a level that could be maintained for the duration of short-term animal exposure studies. The system was operated to generate total constant concentrations of aerosol, while Fe loading varied from 0 to 100 $\mu g/m^3$ in the diluted post-flame gases. The two different concentrations of Fe used in this study were created by adjusting the flow rate of Fe pentacarbonyl vapors mixed with acetylene and ethylene fuels. Particles were collected on carbon grids. The individual size of Fe and soot particles in the exposure chamber was monitored using transmission electron microscopy, while a differential mobility analyzer was used to measure the size distribution of the aerosol. X-ray fluorescence was used to measure the mass concentration of Fe particles ($\mu g/m^3$). The soot mass concentration was found by weighing 25-mm Teflon-coated filters (Teflo, Pall, East Hills, NY) on a microbalance before and after sample collection. The conditions used in the diffusion flame system were reproducible in generating a consistent concentration of Fe throughout the daily 6-h period of exposure.

Iron Mobilization

The amount of Fe mobilized from Fe and soot particles was determined as previously described (Smith et al. 1998), with the following modifications. Particles, on filters, were suspended in 50 m*M* NaCl (1 mg/ml) at a pH of 7.5. The samples were mixed by vortexing for 30 s. Citrate was added to obtain a final concentration of 1 m*M*, and all samples were placed on an orbital shaker for 24 h. The pH was readjusted to 7.5 at regular time intervals throughout the incubation period to prevent alteration in the rates of Fe mobilization. After 24 h, a 1-ml sample was withdrawn and centrifuged at 13,300 × g for 8 min to remove the particles. The amount of Fe mobilized as the citrate:Fe complex in the supernatant was determined, as originally described by Brumby and Massey (1967) for non-heme Fe determination, except that ferrozine (0.4%, w/v) was used instead of 1,10-phenanthroline. This assay uses ferrozine to quantify both Fe(II) and Fe(III) as a result of addition of the reductant ascorbate. The concentration of Fe mobilized by citrate is reported as nanomoles of Fe per milligram of particles.

Animal Exposure

Sprague-Dawley rats at 10 d of postnatal age were exposed via whole-body inhalation to two different concentrations of Fe and soot particles 6 hr/d for 3 d. The average total particle concentration was maintained at 250 $\mu g/m^3$. Iron concentrations were targeted at 30 and 100 $\mu g/m^3$, respectively. Control animals were exposed to filtered air only. Due to the capacity of the particle exposure, eight animals were included in each group with half male and half female rats, and two sets of exposure for each group were performed. Preweanling neonatal rats were exposed to particles away from the dam. Exposure to particles was repeated with the same group of rats at 23 d postnatal age under identical conditions done at 10 d postnatal age. Our rationale for using this dosing scheme based on postnatal age was to subject the lungs of neonatal animals to particle inhalation during critical periods of lung growth where robust cell proliferation occurs in the process of forming new alveoli (postnatal day 10–12) and again during a period of significant alveolar airspace expansion (postnatal day 23–25). Following exposure, samples of bronchoalveolar lavage (BAL) and lung tissues were collected for analysis. At the termination day, the lungs of rats were feasible for conducting BAL procedure. Different sets of exposure were done to allow BAL and lung tissue analyses separately.

Bronchoalveolar Lavage

Preparation of BAL was done following Harrod's protocol with some modifications (Harrod et al., 1998). Within 2 h following the end of particle exposure at 23 d postnatal age, rats were anesthetized using sodium pentobarbital,

50 mg/kg body weight, ip (Nembutal Cardinal Health Inc., Sacramento, CA). The trachea was cannulated, and BAL was performed 4 times sequentially with phosphate-buffered saline (Dulbecco's PBS, Mg^{2+} and Ca^{2+}-free, pH 7.0, GibcoBRL, Grand Island, NY), using a volume equivalent to 28 ml/kg body weight. The 4 aliquots of BAL were centrifuged at 500 × g for 10 min, supernatant was removed, and cell pellets were pooled together for determination of total cell number, viability, and cell differentials. BAL supernatant was aliquoted and stored at −70°C for biochemical analysis.

BAL Fluid (BALF) Analysis for Determination of Lung Injury

Cell pellets were resuspended in 500 µl PBS. Total cell count and viability were determined by trypan blue exclusion with a hemocytometer. A minimum of 1×10^5 cells was used to prepare slides in duplicates by cytospin of 100 µl cell suspension at 600 rpm for 5 min. Cells were stained with Hema 3 (Fisher Scientific Company, Swedesboro, NJ) for the determination of the proportion of macrophages, lymphocytes, and neutrophils. Lactate dehydrogenase (LDH) activity was measured using a colorimetric kit (Sigma-Aldrich, St. Louis, MO) based on the activity of LDH released from damaged cells into the BAL supernatant.

Glutathione

Glutathione (GSH) was measured in BAL supernatant according to the enzymatic method proposed by Tieze and colleagues (1969) and modified by Anderson (1985), using DTNB-oxidized glutathione reductase recycling assay. Reduced GSH was oxidized by DTNB to glutathione disulfide (GSSG) with stoichiometric formation of 5-thio-2-nitrobenzoic acid (TNB). GSSG was reduced to GSH by the action of glutathione reductase and NADPH. The rate of TNB formation was followed at 412 nm and was proportional to the sum of GSH and GSSG present. GSSG was determined after GSH was first derivatized with 2-vinylpyridine.

Antioxidant Power

Antioxidant power was determined in BAL supernatant by ferric reducing/antioxidant power (FRAP) assay according to Benzie and Strain (1999). At low pH, ferric tripyridytrizine (Fe^{3+}-TPTZ) complex was reduced to the ferrous form and was monitored by measuring the change in absorbance at 593 nm. The change in absorbance is directly related to the total reducing power of the electron-donating antioxidants present in the reaction mixture.

Preparation of Lung Homogenate

Twenty-four hours after the last exposure, rats were anesthetized and the lungs were removed immediately from the thorax, frozen in liquid nitrogen, and subsequently homogenized in ice-cold Tris-HCl buffer (25 m*M* Tris, 1 m*M* ethylenediamine tetraacetic acid [EDTA], 10% glycerol, 1 m*M* dithiothreitol [DTT], pH 7.4) with a glass homogenizer. The homogenate was centrifuged at 10,000 × g for 20 min at 4°C. The supernatant was aliquoted and stored at −80°C.

Western Blot Analysis for Ferritin

Western blot analysis was used to determine ferritin levels in the lungs. Briefly, 40 mg protein of lung homogenate was loaded and separated on 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) followed by transfer to a nitrocellulose polyvinylidene fluoride (PDVF) membrane (Bio-Rad, Hercules, CA). The membrane was blocked for 1 h at 25°C in Tris-buffered saline containing 0.1% Tween 20 and 5% nonfat dry milk and probed with primary antibody against human ferritin (rabbit anti-human ferritin polyclonal antibody, Dako, Carpinteria, CA) at a dilution of 1:3000. Secondary antibody (horseradish peroxidase-linked goat anti-rabbit immunoglobulin [Ig] G, Santa Cruz Biotech. Inc., Santa Cruz, CA) was added at a dilution of 1:5000. Purified human liver ferritin (CalBiochem, San Diego, CA) was used as a positive control. The blots were developed by enhanced chemiluminescence detection kit (ECL, AmerSham Pharmacia Biotech, Inc., Piscataway, NJ) with exposure on autoradiography film.

Immunoreactive protein bands were quantified by image densitometry.

ELISA for Proinflammatory Cytokines

Protein levels of proinflammatory cytokines interleukin (IL)-1β and tumor necrosis factor (TNF)-α were measured in lung homogenates by rat enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions (R&D System, Minneapolis, MN). A 1:2 dilution of samples into calibrator diluent provided in the kit was used for the cytokine determination. Quantitation of cytokines was normalized to total protein in the sample.

Statistical Analysis

Student's *t*-test was applied to all data with Staview computer software. All values are presented as mean ± SE. Differences were considered significant at $p < .05$.

RESULTS

Particle Characterization

The mass concentrations of Fe particles generated in combination with soot under 2 conditions were 30 ± 7 μg/m^{3}and 100 ± 28 μg/m^3. Overall mass concentration of the combined Fe and soot for all studies was 250 μg/m^3. The majority of Fe particles generated were Fe oxide in composition with the morphologic appearance of distinct polyhedrons as determined by transmission electron microscopy (TEM) and electron energy loss spectroscopy (EELS) (Figure 1). EELS analysis of particles spectra demonstrated a ratio of Fe to oxygen ranging from 0.5 to 0.7, suggesting the stoichiometric composition of Fe_2O_3. The average particle diameter of mixed Fe and soot was 72 nm with a size distribution ranging from 45 to 110 nm. Since Fe pentacarbonyl was present in the combustion flame, other forms of Fe may have been produced in smaller amounts through interaction with soot.

Iron Mobilization From Particles

Incubation of Fe and soot particles in 50 m*M* NaCl (pH 7.5), in the absence of a metal chelator, did not result in mobilization of Fe. Incubation of Fe and soot particles in the physiologically relevant chelator citrate resulted in Fe mobilization with levels of 37.7 ± 0.9 nmol Fe/mg particles after 24 h. Incubation of blank filters under identical conditions resulted in no detectable mobilization of Fe.

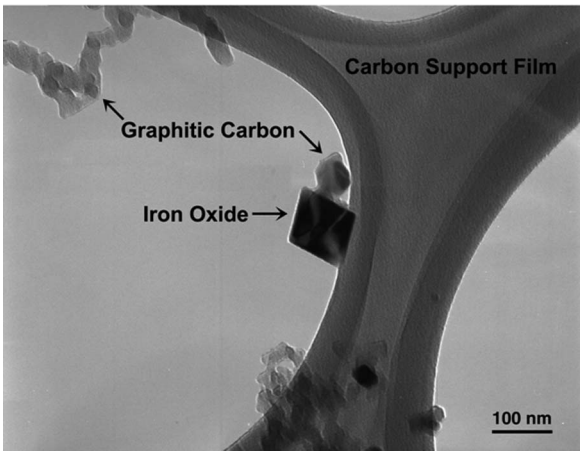

FIGURE 1. Photomicrograph showing carbon support film, graphitic carbon, and iron oxide. Bar = 100 nm.

Cytotoxicity Assessment

To assess the cytotoxicity of inhaled Fe and soot particles, total cell number, cell viability, cell differential, and LDH activity in BAL were used for the determination of acute pulmonary injury (Table 1). There were no significant differences in total cell number or the cell differential between exposure groups. However, exposure to 100 μg/m^3 Fe in combination with soot particles resulted in significant decrease in cell viability and increase in LDH activity. No marked changes were observed in animals exposed to particles containing 30 μg/m^3 Fe combined with soot particles.

Ferritin

Intracellular ferritin levels in homogenized lung tissues were measured using Western blotting following Fe and soot particles exposure. A significant increase in ferritin expression was noted (2.5-fold) in the lungs of animals exposed to particles containing 100 μg/m^3 Fe (Figure 2). No significant difference was present in rats exposed to particles containing 30 μg/m^3 Fe.

TABLE 1. Changes in BAL Fluid Parameters Following Exposure to Iron and Soot Particles in Neonatal Rats

Group	LDH (units/L)	Total cells/ml × 10^5	Cell viability (%)	Macrophages (%)	Lymphocytes (%)	Neutrophils (%)
Control	4.18 ± 1.47	8.23 ± 2.72	94 ± 2.2	98 ± 0.9	1.34 ± 0.89	0.52 ± 0.46
Exposure 1	5.09 ± 0.92	7.38 ± 2.73	92 ± 2.6	98 ± 0.6	0.99 ± 0.58	0.80 ± 0.57
Control	4.09 ± 1.83	16.93 ± 10.16	81 ± 6.1	99 ± 0.5	0.56 ± 0.44	0.26 ± 0.33
Exposure 2	6.96 ± 2.60*	12.74 ± 7.76	66 ± 17.0*	99 ± 0.2	0.41 ± 0.23	0.27 ± 0.17

*Note.*Exposure 1: iron = 30 μg/m^3 with addition of soot to maintain total mass concentration as 250 μg/m^3. Exposure 2: iron = 100 μg/m^3 with addition of soot to maintain total mass concentration as 250 μg/m^3. Values indicate mean ± SE of eight rats per group. Asterisk indicates significant difference from controls at $p < .05$.

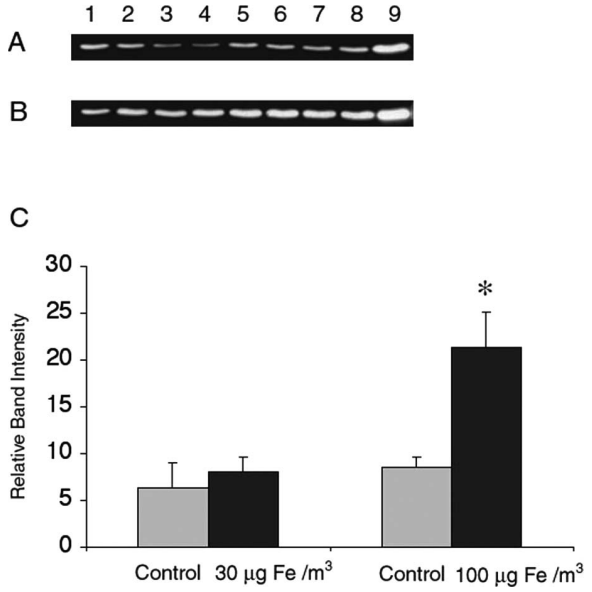

FIGURE 2. Western blot analysis of ferritin expression in rat lung homogenates. (A) Exposure to particle mixture with 30 μg /m^3 of iron. (B) Exposure to particle mixture with 100 μg /m^3 of iron. Lanes 1 to 4: control animals; lanes 5 to 8: exposure animals; lane 9: positive control with purified human liver ferritin. (C) Relative band intensity of ferritin expression by imaging densitometry. Asterisk indicates significant difference from control ($p < .05$).

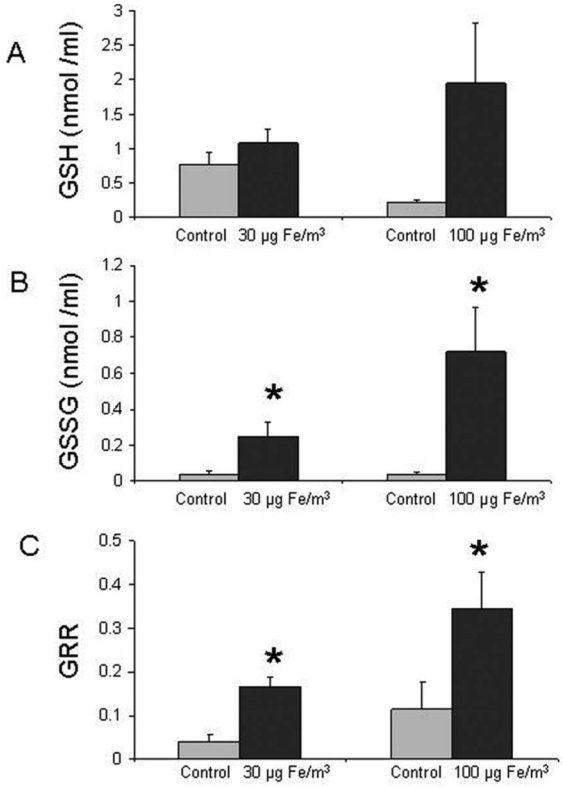

FIGURE 3. Glutathione redox status in bronchoalveolar lavage fluid (BALF). GSH = reduced glutathione; GSSG = glutathione disulfide; GRR = GSSG/(GSH+GSSG), glutathione redox ratio. Data are presented as mean ± SE ($n = 8$ per group). Asterisk indicates significant difference from control ($p < .05$).

Oxidative Stress

Reduced glutathione (GSH), oxidized glutathione (GSSG), the ratio of GSSG/(GSH+GSSG) or glutathione redox ratio (GRR), and ferric/reducing antioxidant power (FRAP) were used as markers of oxidative stress. There were no significant differences in GSH levels between exposure groups. However, exposure to both 30 and 100 μg/m^3 Fe-containing particles resulted in a significant elevation in GSSG and GRR (Figure 3) as well as a significant reduction in antioxidant power (Figure 4).

Proinflammatory Cytokines

Proinflammatory cytokines IL-1β and TNF-α in lung tissues were measured as markers of inflammatory response. Exposure to particles containing 100 μg/m^3 Fe was associated with a significant increase in IL-1β (Figure 5). No significant difference in TNF-α levels was found between groups.

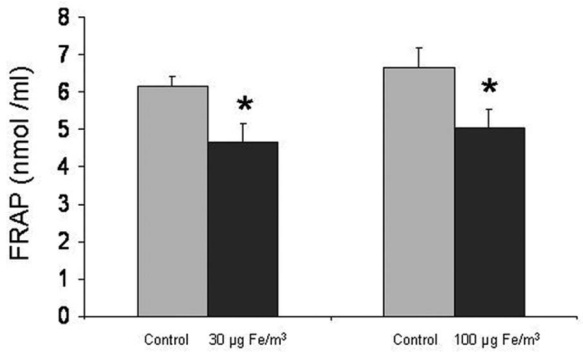

FIGURE 4. Changes in ferric/reducing antioxidant power (FRAP) in BALF. Data are presented as mean ± SE (n = 8 per group). Asterisk indicates significant difference from control ($p < .05$).

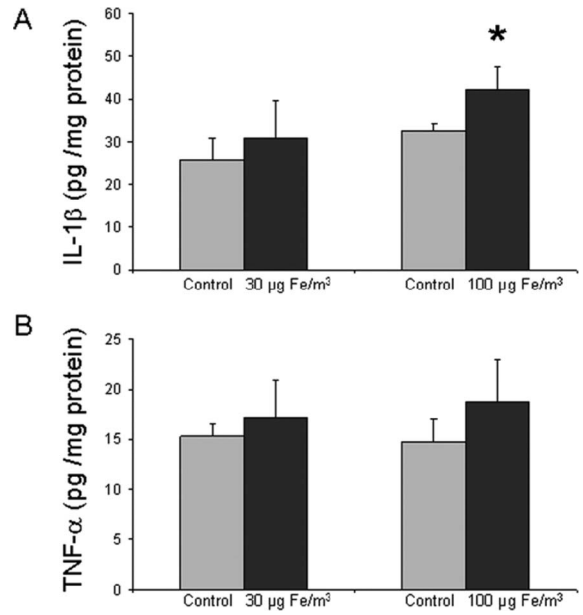

FIGURE 5. Effect of iron and soot exposure on protein levels of cytokines (A) IL-1β and (B) TNF-α in lung homogenates. Values are mean ± SE (n = 6 per group): Asterisk indicates significant difference from control ($p < .05$).

DISCUSSION

Exposure to environmental toxicants during periods of rapid lung development has the potential to significantly affect overall growth and function of the respiratory system (Pinkerton & Joad, 2000; Pinkerton et al., 2004). Children may be especially susceptible to air pollutants since the relative deposition of respirable particles is increased compared with adolescents and adults (Bennett & Zeman, 1998). The effects of ambient PM have been studied and characterized to some extent in adults, but little is known about effects in the developing lung. The present study provides new insights that exposure to ultrafine Fe and soot particles results in lung injury, oxidative stress, and inflammatory response in a dose-dependent pattern in neonatal rats.

Although 24-h average $PM_{2.5}$ concentrations above 65 mg/m^3 are relatively rare in the eastern United States, those concentrations are more prevalent in California, reaching over 150 mg/m^3 (24-h average) in winter in some cities (Ostro, 1995). In the United States, the proportion of Fe may be as high as 16% as measured in Phoenix (U.S. EPA, 2002). Therefore, the particle concentration and compositions used in the present study are environmentally relevant.

Iron is essential for metabolic processes. However, increased availability of Fe may mediate the production of reactive oxygen species (ROS) via the Fenton reaction and may induce oxidative injury and cellular toxicity. Ferritin is an Fe storage protein in the cytoplasm of cells and responsible for the regulation of intracellular Fe (Crichton & Charloteauz-Wauters, 1987; Harrison & Arosio, 1996). Increased synthesis of ferritin reflects an increase in the storage capacity of free Fe (Cermak et al., 1993) and is indicative of bioavailable Fe following exposure to Fe-containing particulate (Fang & Aust, 1997). The current study revealed that a significant induction of ferritin occurred in lungs of neonatal rats exposed to Fe-containing particles at 100 µg/m^3, but not at 30 µg/m^3, in combination with soot particles. The amount of Fe mobilized from Fe and soot particles by citrate in the present study is approximately 2.2-fold greater than that reported for diesel exhaust particulate (Aust et al., 2002), and the amount of Fe mobilized from Utah coal fly ash with a diameter less than 2.5 µm was approximately 1.5-fold higher than from Fe and soot particles (Smith et al., 1998). Thus, the mobilization of Fe from Fe and soot particles is mid-range for what has been reported for other particles.

Smith et al. (1998) demonstrated a direct correlation between the amount of ferritin

induced in human lung epithelial (A549) cells treated with coal fly ash and the amount of Fe mobilized from these particles by citrate in the absence of cells. However, mobilization of Fe by citrate did not correlate with total Fe content of coal fly ash (Smith et al., 1998) or urban PM (Smith & Aust, 1997). Thus, simply determining the amount of Fe present in particles is not likely to allow prediction of bioavailability of Fe. The amount of Fe mobilized from particles is more likely due to factors including the form of the Fe or the surface area of the particles. Bioavailable Fe in urban PM (Smith & Aust, 1997), crocidolite asbestos (Lund & Aust, 1992), and silicates (Hardy & Aust, 1995) was demonstrated to be responsible for catalyzing the formation of ROS. In the present study, the significant induction of ferritin in animals exposed to particles containing 100 μg/m^3 Fe suggests that at least part of the Fe from ultrafine Fe and soot particles deposited in the lungs of neonatal rats is bioavailable. Possible mechanisms accounting for bioavailable Fe following particle exposure may be due to potential interaction between Fe and soot particles leading to reduction of Fe oxide, or enhancement of particle deposition in the lungs and uptake by cells. In support of this possible mechanism is our previous study, which demonstrated that although exposure to Fe or soot particle alone did not induce biological effects, significant oxidative responses in rats exposed to a mixture of these particles were observed, illustrating a synergistic interaction between Fe and soot particles (Zhou et al., 2003).

Previously Zhou et al. (2003) reported that exposure of adult rats to the combination of Fe and soot particles induced pulmonary oxidative stress and IL-1β elevation, effects not associated with decrease in cell viability and increase in LDH activity. Data from our present study revealed the vulnerability of developing lung to the exposure of environmentally relevant concentrations of these particles, as evidenced by the induction of oxidative stress, IL-1β elevation, and lung injury. Oxidative stress was observed in neonatal rats exposed to both 30 and 100 μg/m^{3}of Fe in combination with soot particles, as demonstrated by a significant increase in oxidized glutathione (GSSG) and elevation in GRR, associated with a decrease in antioxidant power. Furthermore, animals exposed to 100 μg/m^3 Fe in the presence of soot demonstrated lung injury in the form of decreased cell viability and increased LDH activity, as well as elevation of proinflammatory cytokine IL-1β. It is postulated that higher Fe concentration (100 μg/m^3) in combination with soot leads to greater bioavailability of Fe, resulting in severe cellular responses including oxidative injury and inflammatory response. Indeed, Smith et al. (2000) reported a direct relationship, above a threshold level of bioavailable Fe, between levels of the proinflammatory cytokine interleukin-8 (IL-8) and bioavailable Fe in A549 cells treated with coal fly ash. Induction of this cytokine was inhibited by antioxidants, suggesting a role of ROS. In addition, pretreatment of coal fly ash with the metal chelator to remove mobilizable Fe prior to treatment of A549 cells resulted in attenuation of IL-8 production to levels similar to those in untreated cells, suggesting a role of Fe in increased cytokine production. The observations in our current study, that exposure to Fe and soot particles induced elevation in proinflammatory cytokine IL-1β, but not in TNF-α, are consistent with the results of previous Fe-containing particle studies (Broeckaert et al., 1997; Dreher et al., 1997; Kodavanti et al., 1997; Zhou et al., 2003). It is noteworthy that although Fe and soot particle exposure resulted in decreased in cell viability and increased LDH activity in BAL fluid, although no significant change in inflammatory cell count was found. A possible explanation for this observation is that exposure to Fe and soot particles induced cell death of local bronchoalveolar cells and LDH elevation in the absence of the recruitment of inflammatory cells at the examination time point in our study; the recruitment of inflammatory cells might be influenced by the concentrations of exposures as well as the phases of inflammatory responses.

In conclusion, these findings suggest that exposure to the mixture of Fe and soot particles induces oxidative injury and inflammatory response in the lungs of neonatal rats in a dose-dependent pattern. The responses

observed from the present study are associated with the bioavailable Fe from inhaled particle mixtures.

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