Journal of Toxicology and Environmental Health, Part A
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713667303

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Online publication date: 07 January 2011

To cite this Article Rengasamy, A. , Barger, M. W. , Kane, E. , Ma, J. K. H. , Castranova, V. and Ma, J. Y. C.(2003) 'Diesel Exhaust Particle-Induced Alterations of Pulmonary Phase I and Phase II Enzymes of Rats', Journal of Toxicology and Environmental Health, Part A, 66: 2, 153 — 167
To link to this Article: DOI: 10.1080/15287390306403
URL: http://dx.doi.org/10.1080/15287390306403

Journal of Toxicology and Environmental Health, Part A, 66:153–167, 2003

1528-7394/03 $12.00 + .00
DOI: 10.1080/15287390390155813

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DIESEL EXHAUST PARTICLE-INDUCED ALTERATIONS OF PULMONARY PHASE I AND PHASE II ENZYMES OF RATS

A. Rengasamy

Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, West Virginia, and National Personal Protective Technology Division, National Institute for Occupational Safety and Health, Pittsburgh, Pennsylvania, USA

M. W. Barger, E. Kane

Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, West Virginia, USA

J. K. H. Ma

School of Pharmacy, West Virginia University, Morgantown, West Virginia, USA

V. Castranova, J. Y. C. Ma

Health Effects Research Laboratory, National Institute for Occupational Safety and Health, Morgantown, West Virginia, USA

Although diesel exhaust particles (DEP) are known to produce pulmonary disorders, the xenobiotic metabolic pathways associated with DEP detoxification and bioactivation remain unclear. In this study, the effect of acute exposure of DEP on phase I and phase II enzymes of rat lung was investigated. Intratracheal administration of DEP produced an induction of cytochrome P-450 (CYP) 1A1 enzyme protein and activity at 1 d postexposure, with the enzyme level returning to control at 5 d postexposure. On the other hand, carbon black (CB), a particle control, did not show any induction of CYP1A1 protein or enzyme activity. However, both DEP and CB significantly decreased CYP2B1 protein and enzyme activity at 1 d postexposure. The decrease in CYP2B1 enzyme protein and activity by DEP or CB treatment was observed up to 7 d postexposure. DEP and CB treatments also significantly attenuated glutathione S-transferase (GST)-π protein at 1 d postexposure. Both DEP and CB at 35 mg/kg significantly decreased the activities of GST and catalase at 1 and 7 d postexposure. DEP, but not CB, significantly induced quinone reductase (QR) activity at 7 d postexposure. This study suggests that DEP may induce CYP1A1 and QR enzymes via a chemical effect, while the carbonaceous core may be involved in the attenuation of CYP2B1, GST, and catalase proteins and enzyme activities.

Received 1 February 2002; accepted 16 April 2002.

This research was supported in part by a grant from the National Institutes of Health (RO1 HL62630-02) to J. K. H. Ma, West Virginia University.

This work was presented in part at the 40th annual meeting of the Society of Toxicology, San Francisco, CA, 2001.

Address correspondence to Jane Y. C. Ma, PhD, Pathology and Physiology Reseach Branch, Health Effects Laboratory Division, NIOSH, 1095 Willowdale Road, Morgantown, WV 26505, USA. E-mail: jym1@cdc.gov

The use of diesel engines is steadily increasing because of fuel efficiency and low levels of carbon dioxide emission. However, diesel engines are known to emit 30 to 100 times more particulate matter compared to gasoline engines. Particulate matter with aerodynamic diameter 10 μm or less is designated as PM10 and is proposed to be involved in several cardiopulmonary disorders. Diesel exhaust particles (DEP) are constituents of PM10 in the atmosphere and are known to contain various organic compounds, such as polycyclic aromatic hydrocarbons (PAH), nitroaromatic compounds, quinones and aldehydes, and heterocyclic compounds adsorbed onto the carbonaceous core (Schetzle, 1983; Draper, 1986). Certain compounds of the organic components of DEP are mutagenic (Handa et al., 1983) and carcinogenic (Mauderly et al., 1994).

Long-term exposure to DEP has been shown to induce tumor formation in animals, although the mechanism(s) of the DEP-induced carcinogenicity remains obscure. Recent studies show that exposure of rats to DEP by long-term (2 yr) inhalation or intratracheal instillation resulted in hyperplasia of type II epithelial cells as well as adenomas and adenocarcinomas of epithelial cells (Iwai et al., 1997). The carbonaceous core of DEP, which is devoid of organic matter, also showed a slightly positive rate of lung tumor formation (Kawabata et al., 1986). These studies show that both the organic, containing known carcinogens, and the particulate components of DEP are responsible for the observed carcinogenic activity.

Studies on the alteration of phase I and phase II metabolic enzymes in the cellular response to xenobiotic exposure have yielded valuable information concerning the mechanisms of bioactivation and/or detoxification of chemical carcinogens (Whitlock et al., 1996; Guengerich, 2001). Phase I enzymes, which include cytochrome P-450s (CYP), NADPH-ferredoxin oxidoreductase, and nitroreductase, can catalyze oxidative, reductive, and hydrolytic reactions that are necessary to convert lipophilic compounds to water soluble substances. The phase II enzymes, which include glutathione *S*-transferases (GST), epoxide hydrolases, aryl sulfotransferases, or UDP-glucuronyltransferases, function to produce polar, readily excretable conjugates of glucuronides, sulfate esters, or amino acid derivatives. CYP2B1 is the major constitutive P-450 isozyme in the lung, while CYP1A1 is present at a very low basal level. However, CYP1A1 is inducible by many xenobiotics including PAHs. CYP2B1 has been associated with detoxification of benzo[a]pyrene (Smith & Bend, 1980). The induction of CYP1A1 by environmental PAH has been shown to produce reactive arene oxides that covalently bond to cellular components (Conney, 1982). Although the induction of CYP1A1 enzyme levels by xenobiotics is associated with increased risk of toxicity or cancer, an increase in phase II enzyme levels has been shown to impart a protective effect. Among the phase II enzymes, GST plays a critical role in providing protection against electrophiles and products of oxidative stress. These enzymes facilitate the conjugation of glutathione (GSH) with, and subsequent elimination of, electrophilic compounds including quinones and epoxides (Prestera & Talalay, 1995).

The phase I enzyme induction has been shown to play a role in DEP-induced mutagenicity. Sato et al. (2000) reported that DEP exposure significantly

increased CYP1A1 mRNA as well as the mutant frequency in Big Blue rats, suggesting that the induction of CYP1A1 by PAH components of DEP contributes to the increase in mutant frequency. The possibility that the particulate components of DEP may also affect phase I and phase II enzymes has not been studied in detail. DEP are known to produce inflammatory lung injury through particle-induced production of reactive oxygen species (Nel et al., 2001). Studies have shown that DEP can catalyze the generation of hydroxyl and superoxide anion radicals in the presence of electron donors such as cysteine or H_2O_2 (Vogl & Elstner, 1989; Sagai et al., 1993), and can activate alveolar macrophages to release reactive oxygen species (Hiura et al., 2000). The fact that the carbonaceous core of DEP also induces tumor formation in animal models suggests that there may be an interactive effect between these inflammatory responses and carcinogenicity. Although oxygen radicals generated through DEP exposure can induce DNA damage and tumor induction through other pathways, such as via the formation of 8-hydroxydeoxyguanosine (Nagashima et al., 1995), they may also modulate the content of the phase I and phase II enzymes. This is likely since these oxygen radicals exhibit diverse intracellular reactivity.

The aim of the present study was to investigate the acute effects of DEP exposure on phase I and phase II enzymes in rat lungs. Carbon black (CB), which exhibits a size and core composition similar to those of DEP but contains no organic compounds, was used as a particle control. In this manner, the effects of the organic and particulate components of DEP on these xenobiotic enzyme systems were differentiated.

MATERIALS AND METHODS

Preparation of DEP and CB

DEP (National Institute of Standards and Technology, Standard Reference Material 1650, Gaithersburg, MD) or CB particles (Elftex-12 furnace black, Cabot, Boston, MA) were autoclaved and mixed with pyrogen-free sterile saline. The suspensions were sonicated for 5 min using an ultrasonic processor with a micro tip (Heat System-Ultrasonics, Plainview, NY) prior to intratracheal instillation.

Animal Treatments

Specific pathogen-free male Sprague-Dawley (Hla:SD-CVF) rats (~200 g) were purchased from Hilltop Labs (Scottdale, PA). Rats were kept in cages individually ventilated with HEPA-filtered air and housed in an AAALAC-approved facility under controlled environmental conditions and a 12-h light/dark cycle. Rats were provided food and water ad libitum. After a 1-wk acclimatization period, rats were anesthetized with sodium methohexital (35 mg/kg body weight, ip) and placed on an inclined restraint board. The particulate suspension was mixed well and intratracheally instilled one time (0.25 ml) with a curved ball-tipped cannula (18 gauge). The control animals received saline. The rats were sacrificed at different time intervals, and heart and lungs were

removed and perfused with saline. The lungs were homogenized in Tris-HCl buffer (pH 7.4), and microsomes and cytosol were prepared by differential centrifugation.

O-Dealkylation Assay

7-Ethoxyresorufin *O*-dealkylation (EROD) and pentoxyresorufin *O*-dealkylation (PROD) activities were measured following the method of Burke et al. (1994). Briefly, the reaction mixture (3 ml) containing 0.1 *M* phosphate buffer (pH 7.6), microsomal protein, substrate (3 μ*M* 7-ethoxyresorufin or 5 μ*M* pentoxyresorufin), and 25 μ*M* dicumarol was incubated at 37 °C for 1 min. The reaction was initiated by the addition of NADPH (final concentration 480 μ*M*), and the production of resorufin was determined by measuring the increase in fluorescence intensity at an excitation of 530 nm and an emission of 585 nm in a Perkin-Elmer LS50 luminescence spectrometer (Perkin-Elmer, Norwalk, CT). A standard curve was constructed using different volumes (2 to 20 μl) of authentic resorufin (10 μ*M*).

GST Assay

The activity of GST was measured as described by Habig et al. (1974). The reaction mixture (1 ml) containing 0.1 *M* phosphate buffer (pH 6.5), 1 m*M* reduced glutathione (GSH), 1 m*M* 1-chloro-2, 4-dinitrobenzene (CDNB), and lung cytosol (100 μg protein/ml) was incubated at 37 °C, and the increase in absorbance of the GST conjugate was measured using a Shimadzu ultraviolet (UV)-2401 PC spectrophotometer (Shimadzu, Columbia, MD) at 340 nm. GST activity was calculated using extinction coefficient of 9.6 $mM^{-1}cm^{-1}$ (Habig et al., 1974), and the specific activity was expressed as nanomoles per minute per milligram.

Catalase Assay

Catalase activity was determined spectrophotometrically (Shimadzu UV-2401 PC) by following the method of Beers and Sizer (1952). Briefly, the reaction mixture (1 ml), containing 100 m*M* phosphate buffer (pH 7), lung cytosol (50 μg protein/ml), and 5 m*M* H_2O_2, was incubated at 37 °C and the degradation of H_2O_2 at 240 nm ($\Delta\varepsilon = 0.045\ mM^{-1}cm^{-1}$) was monitored. The specific activity was expressed as micromoles per minute per milligram.

Quinone Reductase Assay

Quinone reductase (QR) activity was measured as described previously (Ernster, 1967) with some modifications. The reaction mixture (1 ml), containing 25 m*M* Tris-HCl (pH 7.2), 0.23 mg bovine serum albumin, 5 μ*M* flavin adenine dinucleotide, 0.2 m*M* NADPH, and 14 μg/ml of lung cytosol, was incubated at 37 °C. The reaction was initiated by the addition of 2,6-dichlorophenolindophenol (final concentration 40 μ*M*), and the decrease in absorbance at 600 nm (Shimadzu UV-2401 PC) was monitored for 10 min. The specific activity was calculated from a DCI standard curve and expressed as micromoles per minute per milligram.

Western Blot Analysis

The lung microsomes (100 μg) or cytosol (50 μg) was fractionated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using Novex precast gradient gels (4–12% for CYP enzymes and 4–20% for GST, Invitrogen, Carlsbad, CA). The proteins were transferred to a nitrocellulose membrane. The blot was blocked with 5% nonfat dry milk in 20 m*M* Tris-HCl (pH 7.4) buffer containing 0.15 *M* NaCl and (TBS) for 1 h at room temperature. The blot was washed with TBS 0.1% Tween 20 (TBST) three times (5 min each) and incubated with primary polyclonal anti-CYP1A1 antibody (Xenotech, Kansas City, KS), anti-CYP2B1 antibody (Amersham, Arlington Heights, IL), or anti-glutathione *S*-transferase-π antibody (Calbiochem, San Diego, CA) overnight at 4 °C. The blot was washed three times with TBST and exposed to biotinylated secondary antibody for 1 h at room temperature. After washing, the blot was exposed to streptavidin–horseradish peroxidase for 20 min. The blot was washed four times with TBST and the antigen–antibody complex was detected on a photographic film using ECL reagent (Amersham, Arlington Heights, IL).

Statistical Analysis

Data are presented as means ± standard errors. Comparisons were made using one-way analysis of variance (ANOVA) with means testing by Dunnett's or Tukey's test; $p < .05$ was considered to be significant.

RESULTS

CYP1A1 Protein and Enzyme Activity

The acute effects of intratracheally instilled DEP or CB at different doses (5, 15, or 35 mg/kg) on phase I and phase II enzymes of rat lungs were investigated by determining enzyme activities and protein levels at different post-exposure time intervals. CYP1A1 activity as measured by EROD activity was significantly upregulated by DEP, but not by CB, at 1 d postexposure at 15- and 35-mg/kg doses (Figure 1A). This increase in enzyme activity was shown to coincide with an induction of CYP1A1 protein measured using the Western blot technique. Figure 1B shows that at 1 d postexposure of DEP at all doses there was significantly increased CYP1A1 protein level, while CB treatment produced no significant effect. Although 5 mg/kg DEP did not significantly affect CYP1A1 activity, a significant increase in CYP1A1 protein level was observed at this dose (Figure 1B). The time dependence of the CYP1A1 induction process was determined via measurements of CYP1A1 protein levels and EROD activity in rats sacrificed at different days after DEP or CB (35 mg/kg) exposure. The CYP1A1 protein in rats exposed to DEP was significantly greater than that of the controls at 1 and 3 d postexposure, but decreased to the control levels at 5 and 7 d (Figure 2, A and B). This time course of induction of CYP1A1 protein by DEP correlated with changes in EROD activity at different

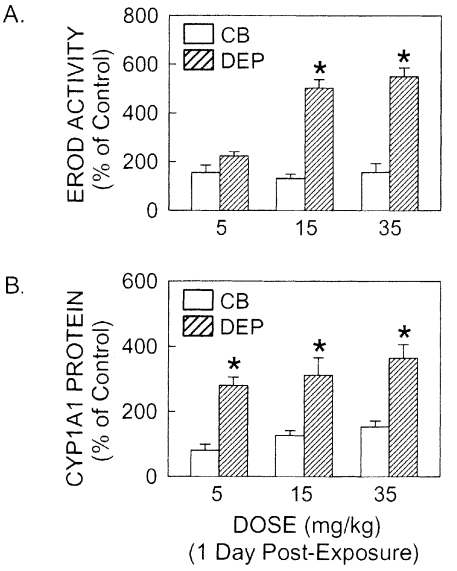

FIGURE 1. Effect of different doses of DEP or CB on the induction of CYP1A1 enzyme activity and protein level. Rats were intratracheally instilled with various doses of DEP or CB; lungs were removed at 1 d post-exposure; and microsomes were prepared. (A) Microsomal *O*-dealkylation of 7-ethoxyresorufin (EROD) was taken as a measure of CYP1A1 activity as described in Materials and Methods. (B) Quantitation of CYP1A1 protein: Microsomal proteins (100 µg protein) were analyzed by Western blot, and CYP1A1 was identified using a polyclonal anti-CYP1A1/2 antibody. CYP1A1 protein was quantitated and normalized for nonspecific protein loading using Eagles Eye II (Strategene, La Jolla, CA) and FluorImager-Imagequant software (Molecular Dynamics, Sunnyvale, CA). Data are from three or more experiments with three or more rats in each group. EROD activity or CYP1A1 protein level was expressed as the percentage of the saline control. CYP1A1 enzyme activity of the saline control was 4.62 ± 0.31 pmol/min/mg. Asterisk indicates a significant difference from the saline control ($p < .05$).

time points (Figure 2C). In contrast, the CB treatment did not produce any significant effect on EROD activity or CYP1A1 protein at any of the time points tested.

CYP2B1 Protein and Enzyme Activity

Contrary to the effects on CYP1A1, both DEP and CB at 15 and 35 mg/kg doses significantly inhibited CYP2B1 activity (PROD) at 1 d postexposure (Figure 3A). Similarly, CYP2B1 protein level was significantly decreased at 1 d postexposure to DEP at 5, 15, and 35 mg/kg doses and postexposure to CB at 15 and 35 mg/kg doses (Figure 3B). The effect of time on the inhibition of CYP2B1 protein by DEP or CB exposure at 35 mg/kg is shown in Figure 4, A and B. This inhibition is associated with a significant decrease in CYP2B1 enzyme activity up to 7 d postexposure (Figure 4C). These results show that DEP or CB had a sustained effect in lowering the enzyme level to exhibit reduced CYP2B1 activity (over 50%).

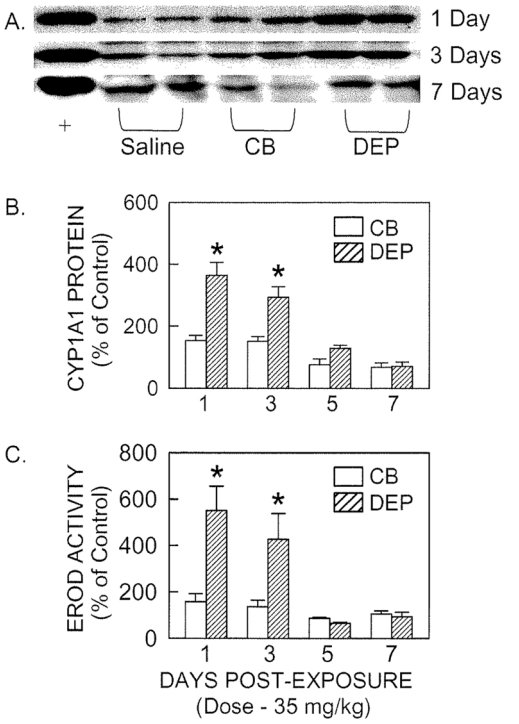

FIGURE 2. Time dependence of the DEP or CB induction of CYP1A1 enzyme activity and protein. Rats were intratracheally instilled with 35 mg/kg of DEP or CB. Lung microsomes were prepared at different postexposure times. (A) Western blot analysis of lung microsomes (100 μg protein) and detection of CYP1A1 using a polyclonal anti-CYP1A1/2 antibody. This blot is representative of four experiments. (B) Summary data for quantitative densitometry as described in Figure 1B. (C) Microsomal *O*-dealkylation of 7-ethoxyresorufin (EROD) activity. Data are from three or more experiments with three or more rats in each group. The CYP1A1 protein and EROD activity were expressed as the percentage of the saline control. CYP1A1 enzyme activity of the saline control was 4.62 ± 0.31 pmol/min/mg. Asterisk indicates a significant difference from the saline control ($p < .05$).

GST-π Protein and Enzyme Activity

The presence of different isoforms of cytosolic GST in rat lungs has been reported previously (Eaton & Bammler, 1999; Hayes & Pulford, 1995). GST-π has been recognized as an important detoxification isozyme in the lungs. In the present study, the effects of intratracheal instillation of different doses of DEP or CB on the total cytosolic enzyme activity and the π isoform protein level of GST were determined. Western blot analysis shows that both DEP and CB at 5, 15, and 35 mg/kg significantly attenuated the GST-π protein levels at 1 d postexposure (Figure 5). Both DEP and CB, at 35 mg/kg, significantly decreased GST activity at 1 and 7 d postexposure (Table 1). GST activity was also significantly inhibited by DEP or CB at 15 mg/kg, but not at 5 mg/kg, at 1 d postexposure (data not shown).

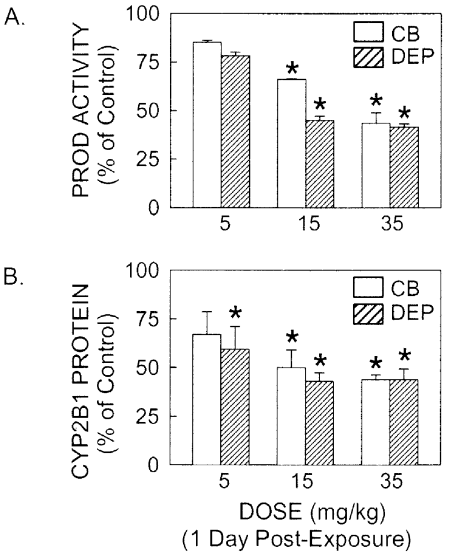

FIGURE 3. Effect of different doses of DEP or CB on the activity and protein levels of CYP2B1. Lung microsomes were prepared from rats exposed to various doses of DEP or CB at 1 d postexposure. (A) Microsomal *O*-dealkylation of pentoxyresorufin (PROD) was taken as a measure of CYP2B1 activity as described in Materials and Methods. (B) CYP2B1 protein quantitation of Western blots as described in Figure 1B. Data are from three or more experiments with three or more rats in each group. PROD activity or CYP2B1 protein level was expressed as the percentage of the saline control. CYP2B1 enzyme activity of the saline control was 6.29 ± 0.45 pmol/min/mg. Asterisk indicates a significant difference from the saline control ($p < .05$).

TABLE 1. Effects of Carbon Black (CB) and Diesel Exhaust Particles (DEP) on the Activities of GST, Catalase, and Quinone Reductase

	GST		Catalase		Quinone reductase	
Postexposure time	CB	DEP	CB	DEP	CB	DEP
1 d	69 ± 3[a]	62 ± 7[a]	70 ± 3[a]	72 ± 4[a]	73 ± 10	105 ± 3
7 d	61 ± 4[a]	70 ± 2[a]	70 ± 9[a]	82 ± 5[a]	102 ± 8	120 ± 4[a]

Note. Rats were intratracheally instilled with DEP (35 mg/kg) and the activities of GST, catalase, and quinone reductase were measured in the lung cytosol at 1 or 7 d postexposure as described in Materials and Methods. The activities of GST, catalase, and quinone reductase were expressed as the percentage of the saline control. The specific activities of saline control of GST, catalase, and quinone reductase were 244 ± 20 nmol/min/mg, 20 ± 1 μmol/min/mg, and 835 ± 89 μmol/min/mg, respectively.

[a] Significantly different from the saline control, $p < .05$.

Catalase Activity

The effect of DEP or CB exposure on rat lung catalase activity was determined at different time intervals. Catalase activity was significantly inhibited by 35 mg/kg DEP or CB at 1 and 7 d postexposure (Table 1). However, the measured catalase activity from rats exposed to 5 or 15 mg/kg DEP or CB was not significantly different from that of the control at 1 d postexposure (data not shown).

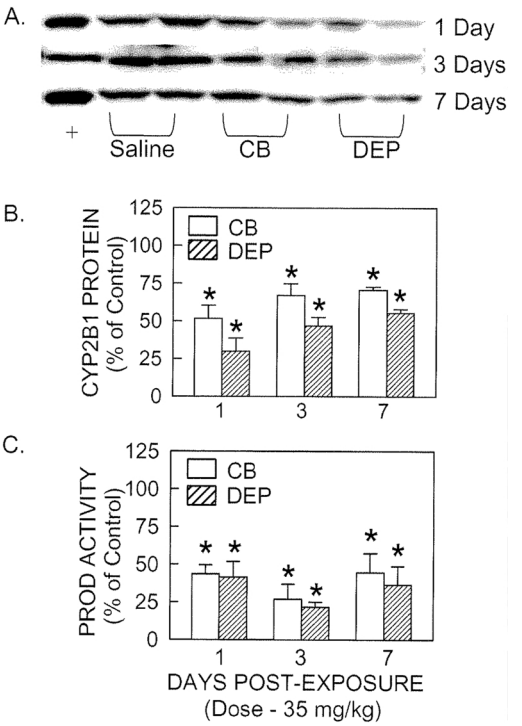

FIGURE 4. Time dependence of the DEP or CB attenuation of CYP2B1 enzyme activity and protein level. Rats were intratracheally instilled with 35 mg/kg of DEP or CB. Lung microsomes were prepared at 1, 3, and 7 d postexposure. (A) Western blot analysis of lung microsomes (100 µg protein) and identification of CYP2B1 protein using polyclonal anti-CYP2B1 antibody. This blot is representative of three experiments. (B) Summary data for quantitative densitometry as described in Figure 1B. (C) Microsomal CYP2B1 activity was measured as *O*-dealkylation of pentoxyresorufin (PROD). Data are from three or more experiments with three or more rats in each group. The CYP2B1 protein and PROD activity were expressed as the percentage of the saline control. CYP2B1 enzyme activity of the saline control was 6.29 ± 0.45 pmol/min/mg. Asterisk indicates a significant difference from the saline control ($p < .05$).

Quinone Reductase Activity

Quinone reductase (QR) activity of lung cytosol from rats exposed to 35 mg/kg of DEP or CB was measured at 1 and 7 d postexposure. DEP treatment resulted in a significant increase in QR activity at 7 d postexposure (Table 1). In contrast to DEP, the CB treatment did not have a significant effect on QR activity at any time point tested.

DISCUSSION

This study was carried out to characterize the effects of DEP exposure on the xenobiotic metabolic pathways that are associated with pulmonary detoxification and bioactivation. Our results show that DEP significantly altered both phase I

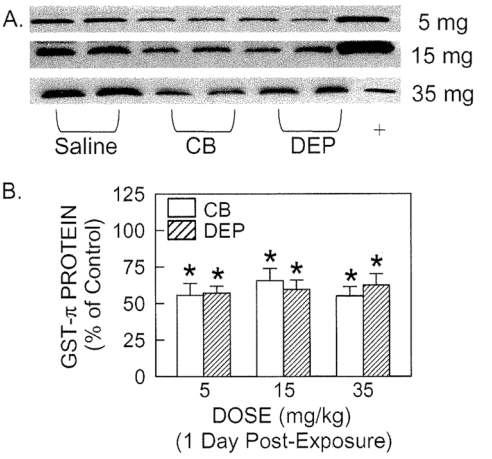

FIGURE 5. Effect of different doses of DEP or CB on GST-π protein. Rats were intratracheally instilled with various doses of DEP or CB; lungs were removed at 1 d postexposure; and cytosol was prepared. (A) Western blot analysis of cytosolic proteins (50 μg) and detection of GST-π using anti-GST-π antibody. This blot is representative of four experiments. (B) Densitometric results of data from three or more experiments with three or more rats in each group. The GST-π protein was expressed as the percentage of the saline control. Asterisk indicates a significant difference from the saline control ($p < .05$).

and phase II enzymes in the lung. Following a single exposure by intratracheal instillation, the induction of CYP1A1 by DEP was maximal at 1 d postexposure, with the enzyme level returning to normal at 5 d postexposure. Since the CYP1A1 activity or protein level in the lung was not affected by CB exposure, it is likely that the DEP effect on this enzyme stems from the organic components adsorbed onto the carbonaceous particulate core. Although less pronounced, DEP, but not CB, also increase the quinone reductase (QR) activity in the lung cytosol. The activities and protein levels of CYP2B1, GST, and catalase in rat lungs were significantly reduced by both DEP and CB exposures, suggesting that the carbonaceous particulate itself also induced changes in the phase I and phase II enzymes. The fact that both DEP and CB, which are known to induce a macrophage respiratory burst and the release of reactive oxygen species, resulted in lowered catalase activity gives rise to a proposal that oxygen radicals may be involved in the reduction of CYP2B1 and GST.

The induction of CYP1A1 observed in the present investigation is supported by results of studies that report that motorcycle exhaust causes the induction of CYP1A1 in rat lung (Ueng et al., 1998) and human hepatic- and pulmonary-derived cell lines (Ueng et al., 2000). The role of the organic components of DEP, including PAH and nitroaromatic compounds, in the activation of CYP1A1 metabolism has been widely studied (Conney, 1982). Yamasaki et al. (2000) showed that several fractions of the organic components of DEP induced the expression of human CYP1A1 gene cloned into *Escherichia coli*. Previous studies also have reported that CYP1A1 is highly inducible by PAH treatment and is typically the most active isoform in total PAH metabolism in rodent liver and

lung (Christou et al., 1987). This suggests that components of DEP, including PAH and nitropyrenes, may be responsible for the induction of CYP1A1 in rat lungs. It should be noted that a benzene–ethanol extract of DEP was tested and gave no appreciable induction of human CYP1A1 in a cloned system (Yamasaki et al., 2000). This may be due to an insufficient amount of DEP extract used in the study or may indicate that the particulate, which serves as a carrier, plays a role in delivering the organic content. The DEP doses (5 to 35 mg/kg) employed in the current study have been found to suppress macrophage functions in the lung (Yang et al., 2001), but these doses are sufficient to induce CYP1A1 in the lung.

Studies have shown that the two members of the CYP1A family, CYP1A1 and CYP1A2, are structurally similar but have different substrate specificity (Ghanayem et al., 2000). The induction of microsomal EROD (CYP1A1) activity and CYP1A1 protein by DEP suggests that DEP may have a high concentration of chemicals that are specific substrates for CYP1A1. Indeed, the major PAHs of the benzene–ethanol extract of DEP are benz[a]anthracene, benzo[a]pyrene, and benzo[ghi]perylene, which have been shown to induce CYP1A1 (Yamasaki et al., 2000). The expression of CYP1A1 may be mediated by the AhR receptors, as revealed from AhR-knockout mouse studies (Ghanayem et al., 2000), suggesting that some of the effects of DEP may be mediated through AhR receptors.

DEP exposure significantly decreased lung microsomal CYP2B1 protein levels with a concomitant reduction in PROD (CYP2B1) activity. This effect was sustained for 7 d. To our knowledge, the effect of intratracheal instillation of DEP on lung CYP2B1 has not been reported. Recent studies have shown that treatment of rats with β-naphthoflavone downregulates CYP2B1 protein and PROD activity in rat lung microsomes as well as in mitochondria, although the mechanism of this effect remains unclear (Bhagwat et al., 1999). On the other hand, Ueng et al. (1998) showed an induction of CYP2B1 protein as well as an increase in PROD activity in motorcycle exhaust-treated rat liver. Taken together, these results suggest that the effect of DEP on CYP2B1 expression is perhaps tissue specific and may depend on the exposure conditions such as route, dose, and exposure duration.

DEP exposure may alter CYP2B1 in the lung via several mechanisms. A sudden increase in toxic load by DEP instillation may overwhelm the detoxification systems and compete for metabolic enzyme activities. Drugs such as cimetidine, which bind directly to the heme iron of the CYP reactive site, and naringenin of grapefruit are able to inhibit phase I enzyme activities (Liska, 1998). Compounds, such as toluene and xylene, which are present in DEP, may downregulate specific CYP450s. It has been reported that exposure of rats to toluene (inhalation) (Furman et al., 1998) or *p*-xylene (ip) (Verschoyle et al., 1993) significantly inhibited lung CYP2B1 activity without affecting CYP1A1 activity. The mechanism by which these agents inhibit CYP2B1 appears to involve the formation of an aldehyde–heme adduct (Raner et al., 1997). Interestingly, Nakajima and Wang (1994) reported that toluene when administered ip induced

both CYP2B1 and CYP1A1 in the liver. The reason for this difference is likely due to the route of toluene administration.

Another mechanism of DEP downregulation of CYP2B1 may involve nitric oxide (NO). NO avidly binds to reactive heme–iron centers of several enzymes and thereby regulates their enzyme activities. Bacterial lipopolysaccharide (LPS) has been shown to downregulate CYP2B1 in rat hepatocytes by an NO-dependent mechanism (Ferrari et al., 2001), which was completely prevented by NO synthase inhibitors. Studies have also shown that LPS downregulates the expression of CYP2B1/2 through the NO-dependent pretranslational regulation of CYP2B1/2 mRNA (Khatsenko et al., 1997). In a previous study, data showed that DEP exposure resulted in a moderate increase in macrophage production of NO (Yang et al., 2001). This suggests that NO may be partly responsible for the DEP downregulation of CYP2B1 reported in the present investigation.

The present study shows that both DEP and CB downregulate CYP2B1 protein and enzyme activity in the lung, suggesting that factors other than the adsorbed organic compounds are involved in this process. Previously, it was demonstrated that both particle types induced the respiratory burst by alveolar macrophages and the release of hydrogen peroxide and superoxide anions (Dong, 1998). In the present study, evidence indicates that these particle exposures were associated with a decrease in catalase activity. It is possible that the reduction of CYP2B1 in the lung associated with particle exposure is mediated through increased production of reactive oxygen and nitrogen species.

The DEP exposure was found to downregulate GST-π protein and enzyme activity in rat lungs. GST-π is well expressed in the lung, while GST-α is present at very low levels (Eaton & Bammler, 1999; Hayes & Pulford, 1995). Thus, a decrease in GST-π protein level, as observed in our study, is expected to reflect a decreased total GST activity. Such a decrease in GST activity produced by DEP agrees with a previous study that showed intratracheal instillation of DEP markedly inhibited GST and other phase II enzymes including SOD, and glutathione peroxidase in a mouse model (Sagai et al., 1993). On the other hand, motor exhaust particles were shown to increase GST activity in lung cytosol when administered through inhalation, but not through intratracheal or intraperitoneal injection (Ueng et al., 1998). One possible explanation for these results may lie in the differences in the dose, particle distribution, or particle clearance produced by the various methods of exposure (Osier & Oberdorster, 1997).

Studies have shown that GST-π is inactivated by the presence of H_2O_2 through formation of disulfide linkage between reactive cysteine sulfhydryl residues (Shen et al., 1991). This in part may explain the effects of DEP on GST activity. Catalase controls the H_2O_2 level in vivo; a decrease in catalase activity by DEP or CB could increase cellular H_2O_2, thus resulting in lowered GST activity. There is in fact a direct inhibitory effect of DEP on catalase activity, which supports the concept of H_2O_2-mediated decrease in GST activity. DEP inhibited the activity of isolated catalase from various cell types (Mori et al., 1996).

In cell cultures pretreated with catalase, DEP-induced cytotoxicity was consistently reduced (Hirafuji et al., 1995).

The DEP exposure was associated with a significant increase in quinone reductase (QR) activity in the lung. This may be attributed to the possible presence of electrophilic compounds and phenolic antioxidants in DEP. Such compounds have been shown to induce QR as a chemoprotective strategy in biological systems (Talalay & Prochaska, 1987; Wattenberg, 1983; Prestera & Talalay, 1995). In comparison, CB, which does not contain adsorbed organic compounds, did not alter QR activity in the rat lung.

The present study shows that the action of DEP on pulmonary phase I and phase II enzymes is mediated through both chemical and particulate effects. Our laboratory has reported previously that both DEP and CB induced pulmonary inflammatory responses due to a particle effect, while the chemical effect of DEP mediated the suppression of LPS-induced immune response (Yang et al., 1999). Using CB as a particle control to provide identical characteristics in core material, size distribution, and surface area, this study demonstrates that DEP induced CYP1A1 and QR activity in the lung via a chemical effect and inhibited the CYP2B1, GST, and catalase pathways via a particulate effect. While the significance of each of these changes induced by DEP on pulmonary carcinogenicity remains to be studied, repeated evidence indicates that both DEP and CB produce significant and concentration-dependent increases in tumor induction in the lung (Nikula et al., 1995; Mauderly et al., 1994). This suggests that both the organic and particulate components play an important role in DEP-mediated pulmonary toxicity. The induction of CYP1A1 by the organic component is transient in short-term exposure, but may be involved in the process of carcinogenesis under chronic exposure conditions. Equally significant, however, is the fact that both DEP and CB exhibit a significant and sustained effect on the reduction of CYP2B1, GST, and catalase enzymes in the lung, which may indeed lead to severe impairment of lung metabolism.

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