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Effects of exposure to diesel exhaust particles (DEP) on pulmonary metabolic activation of mutagenic agents

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Abstract

Exposure of rats to diesel exhaust particles (DEP) or carbon black (CB) has been shown to induce time-dependent changes in CYP1A1and CYP2B1 in the lung. The present study evaluated the role of these metabolic enzymes on the pulmonary bioactivation of mutagens. Male Sprague–Dawley rats were intratracheally instilled with saline (control), DEP or CB (35 mg/kg body weight) and sacrificed at 1, 3, or 7 days post-exposure. Both control and exposed lung S9 increased the mutagenic activity of 2-aminoanthracene (2-AA), 2-aminofluorene (2-AF), 1-nitropyrene (1-NP), and the organic extract of DEP (DEPE) in Ames tests with *Salmonella typhimurium* YG1024 in a dose-dependent manner. Lung microsomes prepared form control or particle-exposed S9, but not cytosolic protein, activated 2-AA mutagenicity. Compared to saline controls, CB-exposed S9 was a less potent inducer of 2-AA mutagenicity at all time points, whereas DEP-exposed S9 was less potent than control saline at 3 and 7 days but not 1 day post-exposure. At 3 days post-exposure, DEP- or CB-exposed lung S9 did not significantly affect the mutagenicity of DEPE or 1-NP, when compared to the controls. The mutgenicity of 2-AA, 2-AF, 1-NP, and DEPE were significantly decreased in the presence of inhibitors for CYP1A1 (α-naphthoflavone) or CYP2B (metyrapone), but markedly enhanced by CYP1A1 or CYP2B1 supersomes with all the cofactors, suggesting that both CYP1A1 and CYP2B1 were responsible for mutagen activation. These results demonstrated that exposure of rats to DEP or CB altered metabolic activity of lung S9 and S9 metabolic activity dependent mutagen activation. The bioactivation of mutagens are metabolic enzyme- and substrate-specific, and both CYP1A1 and CYP2B1 play important roles in pulmonary mutagen activation.

Keywords: Diesel exhaust particles; Carbon black; Mutagenicity; CYP1A1; CYP2B1

1. Introduction

The use of diesel engines is steadily increasing due to fuel efficiency, and efforts to lower emissions from

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diesel engines have been made for the past 20 years [1]. Diesel exhaust emissions contains fine particulate matter ($PM_{2.5}$) composed of carbon-based particles, which have adsorbed various organic compounds, including polycyclic aromatic hydrocarbons (PAHs), quinones, and nitro-PAHs [2]. Many of these organic compounds associated with diesel exhaust particles (DEP) are known to be mutagenic and carcinogenic [3,4]. Studies have shown that DEP have a mass median aerodynamic diameter in the range of 0.05–1.00 μm (mean: 0.2 μm), which is a size distribution conducive to particle penetration and deposition in the alveolar region of the lung [5]. In addition, numerous studies have shown that DEP represent a major component of ambient PM and may pose a significant risk for the development of lung cancer after long-term, high dose exposures in occupational settings [6–8].

DEP are known to cause pulmonary neoplasms in the lung of rats and mice [9,10]. PAH-derived DNA adducts, which may play a role in DEP-mediated mutagenicity and carcinogenicity, were found in rats after a short-term (12 weeks) exposure to DEP [11]. Elevated levels of PAH-derived DNA adducts have also been observed in white blood cells of humans following DEP exposure [12]. However, studies have shown that carbon black (CB) particles, which resemble the carbonaceous core of DEP but are devoid of the organic compounds, also induce lung tumor formation in long-term inhalation exposure experiments using a rat model [3,13], although the frequency of neoplasms was slightly lower among CB- than DEP-exposed rats. These studies suggest that the particulate component may be involved in DEP-induced carcinogenic activity.

Although the mechanism for carcinogenisis of DEP is not yet clear, both inflammatory and carcinogen-mediated pathways have been proposed. The inflammatory mechanism, which is induced by particulate matter, may be predominant under high-level exposure conditions. Factors that contribute to a tumor-promoting effect may involve inflammatory injury, cell proliferation, impairment of lung clearance, and generation of reactive oxygen species (ROS) [14]. On the other hand, a genotoxic mechanism may be through the adsorbed organic carcinogens and mutagens on DEPs [15]. PAHs, including direct- and indirect-acting mutagenic and carcinogenic compounds, are thought to play a key role in such a process [16]. It should be mentioned that PAH carcinogens often require bioactivation by cytochrome P450 (P450 or CYP) enzymes before exhibiting toxic and/or carcinogenic effects. Recent studies have demonstrated that these enzymes are primarily responsible for the generation of intracellular ROS induced by DEP exposure [17], suggesting that P450 enzymes may contribute to both the inflammatory and genotoxic mechanisms of DEP carcinogensis.

The P450 monooxygenase-dependent system comprises a superfamily of enzymes that collectively catalyze the biotransformation of a great variety of xenobiotic and endogenous compounds into water soluble compounds that can be eliminated from the body. However, in some cases, these metabolic reactions form carcinogenic intermediates [18]. The lung is a primary target for airborne toxic substances, and a number of studies have been focused their investigation on the role of pulmonary microsomal P450s in carcinogenesis and/or xenobiotic metabolism [19–21]. Recent studies from our laboratory have shown that DEP exposure has a profound effect on the pulmonary cytochrome P450 content. In particular, exposure to DEP resulted in a transient induction of CYP1A1 that peaked at 1 day but returned to the basal level at 7 days post DEP exposure. CYP2B1, the major constitutive isoenzyme in the rat lung, was consistently decreased by DEP over a 7-day post-exposure time period. In comparison to DEP, CB exposure did not induce CYP1A1 but resulted in a sustained decrease in the pulmonary content of CYP2B1 [22]. It has been reported that CYP1A1 is the major isozyme responsible for the metabolism of PAHs leading to the formation of bioactive intermediates, DNA adducts, and increased risk of toxicity or cancer [23]. CYP2B1 is the predominant form of cytochrome P-450 constitutively present in the rat lung [24,25]. Stickney et al. [26] proposed that CYP1A1 is responsible for the activation of benzo[a]pryene (B[a]P) mutagenicity, whereas CYP2B1 plays a role in B[a]P detoxication. Since both DEP and CB exposures alter the distribution of pulmonary P450 isozymes, it is reasonable that these particles may affect the bioactivation/detoxification of inhaled xenobiotic agents. In order to gain more insight into the roles of lung CYP1A1 and CYP2B1 in the pulmonary mutagenic responses, the present study characterized the effect of lung metabolic enzymes on the activation of various mutagens, using the Ames

test. The effect of changes in lung metabolic enzymes in response to DEP or CB exposure on this mutagenic activity was also determined.

2. Materials and methods

2.1. Chemicals and reagents

The following chemicals were purchased from Sigma Chemical Co. (St. Louis, MO): β-nicotinamide adenine dinucleotide phosphate (NADP), glucose-6-phosphate, glucose-6-phosphate dehydrogenase, L-histidine, D-biotin, bacteriological agar, D-(+)-glucose, potassium phosphate, magnesium sulfate, citric acid monohydrate, sodium ammonium phosphate, 2-aminoanthracene (2-AA), 2-aminofluorene (2-AF), B[a]P, 1-nitropyrene (1-NP), α-naphthoflavone (α-NF), metyrapone, and dimethyl sulfoxide (DMSO). Oxoid nutrient broth No. 2 was obtained from Oxoid Inc. (Ogdensgurg, NY). Aroclor 1254-induced rat liver S9 was purchased from Molecular Toxicology Inc. (Boone, NC). Diesel particulate extract (DEPE, Standard Reference Material 1975) was obtained from the National Institute of Standards and Technology (Gaithersburg, MD).

2.2. Preparation of DEP and CB

DEP (National Institute of Standards and Technology, Standard Reference Material 2975) and 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.

2.3. Animal exposure

Specific pathogen-free male Sprague–Dawley (Hla:SD-CVF) rats (∼200 g) were purchased from Hilltop Labs Animals (Scottdale, PA). Rats were kept in ventilated polycarbonate cages on Alpha-Dri virgin cellulose chips and hardwood Beta-chips as bedding, and provided HEPA-filtered air, autoclaved Prolab 3500 diet, and tap water ad lib. The animal facilities are specific pathogen-free, environmentally controlled, and AAALAC-accredited. After a 1 week acclimatization period, rats were anesthetized with sodium methohexital (35 mg/kg body weight, i.p.) and were intratracheally (i.t.) instilled with a single dose of DEP or CB particulate suspension (35 mg/kg body weight) or an equal volume of saline as control. The rats were sacrificed at 1, 3, or 7 days post-exposure.

2.4. Preparation of pulmonary subcellular fractions

Animals were anesthetized with sodium pentobarbital (0.2 g/kg body weight, i.p.) and exsanguinated by cutting the renal artery. S9, cytosolic and microsomal fractions were prepared from lung homogenates. Briefly, the heart and lung were removed en bloc. The lungs were perfused with saline to remove blood cells and homogenized in a volume of Tris–HCl buffer (pH 7.4) equivalent to four times the lung weight. S9, microsomes, and cytosol were prepared by differential centrifugation of lung homogenate. The microsomes were resuspended in the homogenizing buffer. The protein concentration of S9, microsomes, and cytosol was determined using a BCA protein assay kit with bovine serum albumin as the standard (Pierce, Rockford, IL). Sterility of the pulmonary subcellular fractions was determined by plating 0.1 ml of S9, microsomes or cytosol on minimal agar containing histidine and biotin.

2.5. Salmonella mutagenicity assay

Prior to use in a mutagenicity assay, activation mixtures were freshly prepared according to Maron and Ames with minor modification [27]. The activation mixtures contained a lung enzyme preparation of S9, microsomes or cytosol, cofactors (5 mM glucose-6-phosphate and 4 mM NADP), and the $MgCl_2$–KCl salt solution. Activation mixtures containing S9 protein were standardized to 25, 50, 100, and 200 μg/plate. The results showed that the cytosolic protein accounting for 80.3 ± 3.0% of S9 protein. For this reason, the activation mixtures containing cytosolic protein was standardized to 20, 40, 80, and 160 μg/plate and the microsomal protein was standardized to 5, 10, 20, and 40 μg/plate to the amount of cytosolic or microsomal protein present in S9. When microsomes served as the activation system, the mixture was supplemented with glucose 6-phosphate dehydrogenase (1 unit/plate).

Salmonella typhimurium YG1024 was used as the tester strain for the Ames test. 2-AA, 2-AF, 1-NP, DEPE, and B[a]P were dissolved in DMSO and used as substrates, while DMSO was used as the negative control. The Ames test was performed using the microsuspension assay, a modification of the preincubation method [28]. Briefly, *S. typhimurium* YG1024 was grown overnight at 37 °C in Oxoid nutrient broth No. 2 and was harvested by centrifugation at 1400 × *g* for 20 min at 4 °C and resuspended to yield a cell concentration of approximately 2.5–5 × 10^9/ml. The 10 μl of substrates containing 2-AA (0.015 μg/plate), 2-AF (0.1 μg/plate), 1-NP (0.03 μg/plate), DEPE (0.01 μg/plate) or B[a]P (10 μg/plate), 65 μl of activation mixtures (described in the previous paragraph), and 25 μl of concentrated overnight bacteria were placed in test tubes and incubated for 30 min at 37 °C on a rotary shaker. For positive controls, Aroclor 1254 induced rat liver S9 (protein concentration of 40 mg/ml at 10% (v/v) in S9 mix) was used as the source of activating enzyme. After the preincubation, 2.4 ml of top agar containing 0.05 mM each of histidine and biotin was added to each sample, and the mixtures were poured onto Vogel–Bonner minimal agar plates. Plates were inverted and incubated at 37 °C for 72 h. The revertant colonies were hand-counted, and the results were expressed as the number of revertant colonies per plate. The background lawn of each sample was examined by light microscopy to monitor the cytotoxicity. All assays were performed on duplicate plates and each assay was repeated three times using different animals.

An inhibitor of CYP1A1 (α-NF, 1 μM/plate) or CYP2B1 (metapyrone, 10 μM/plate) was added to the reaction mixtures in one set of Ames test experiments to monitor the involvement of CYP1A1 and CYP2B1 in lung S9 metabolic activity-dependent mutagenicity.

The mutagenicity of 2-AA, 2-AF, B[a]P, DEPE, and 1-NP was also determined using commercially available rat CYP1A1 and CYP2B1 supersomes to establish the role of CYP1A1 and CYP2B1 in the metabolic activation of these mutagens. Rat CYP2B1 + P450 reductase + cytochrome b_5 SUPERSOMES, and rat CYP1A1 + P450 reductase SUPERSOMES were purchased from BD Biosciences (Woburn, MA). These assays were carried out according to the manufacturer's instructions.

2.6. Statistical analysis

All *Salmonella* assays were performed on duplicate plates and each assay was repeated three times with S9, microsomes, and cytosols isolated from different animals. Spontaneous revertants were subtracted from the number of revertants obtained for each assay. The results were expressed as means ± S.E. Paired Student's *t*-tests were performed to compare the activating capability of different mutagens by lung S9, microsomes or cytosol from DEP- or CB-exposed rats to the saline control. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Effects of DEP or CB exposure on lung S9-mediated 2-AA and 2-AF mutagenicity

Recent studies from our laboratory have shown that exposure of rats to DEP and CB resulted in altered phase I and phase II enzymes in the rat lung [22]. The current study examines the potential effects of DEP or CB exposure on rat lung S9-mediated bioactivation of 2-AA mutagenicity in *S. typhimurium* YG 1024. Fig. 1 shows that the enhancement of 2-AA mutagenicity requires lung S9 and has a dose-dependent relationship. This enhancement was abolished when the S9 proteins were heat-inactivated (data not shown). The results show that DEP-exposed S9 activated 2-AA mutagenicity was similar to the controls at 1 day post-exposure. However, this S9-dependent enhancement of 2-AA mutagenicity was reduced to less than control with S9 obtained from rat lungs at 3 and 7 days post-exposure to DEP. Fig. 1 also shows that lung S9 isolated form rats exposed to CB significantly decreased S9-dependent activation of 2-AA mutagenicity, when compared to the controls at all time points.

Fig. 2 shows the comparative involvement of the microsomal and cytosolic proteins in the activation of 2-AA (Fig. 2A) and 2-AF (Fig. 2B) mutagenicity by S9 from saline-exposed rats, at 3 days post-exposure. These results show that the microsomal fraction caused a small but significant enhancemtn of 2-AA and 2-AF mutagenicity, whereas the cytosol showed little effect on the activation of these mutagens. The dose-dependent relationship on the microsome-mediated 2-AA and 2-AF activation followed a pattern similar to

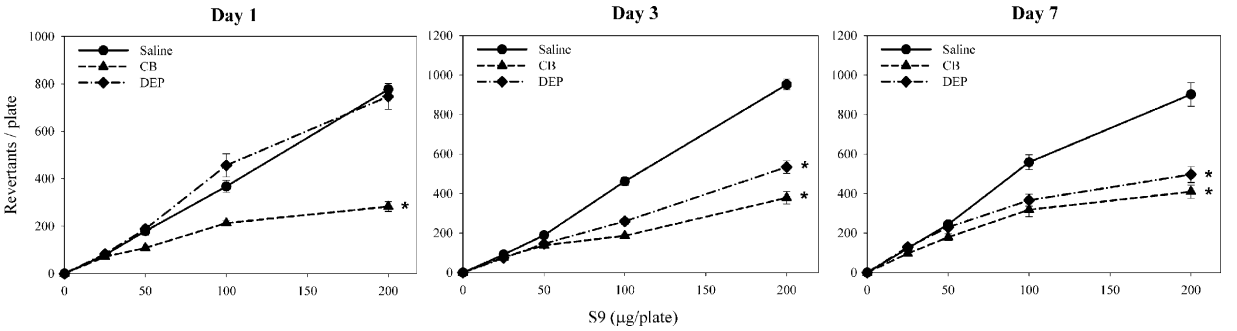

Fig. 1. Metabolic activation of 2-AA by lung S9 from saline-, CB- or DEP-exposed rats at 1, 3, and 7 days post-exposure. The mutagenicity of 2-AA was monitored using *Salmonella typhimurium* YG1024 in the Ames test. The reaction mixture contained 2-AA (0.015 μg/plate) in the presence of various concentrations of S9 as the metabolic activating system. All assays were performed on duplicate plates and each assay was repeated three times using different animals. Each point represents means ± S.E. from three different animals after subtraction of the spontaneous revertants of 45 ± 4/plate. (*) Significantly different from the corresponding control, $p < 0.05$.

that of the lung S9. In addition, Fig. 2 also shows that microsomal enzymes of lung S9 were more involved in 2-AF activation than 2-AA. It should be mentioned that the enhancing effect by intact S9 is significantly greater than the combined effects of the microsomal and cytosolic fractions, suggesting that the cytosol may also contain factors that may enhance the activity of the microsomal enzymes.

3.2. Roles of CYP1A1 and CYP2B1 in mutagenic activation

The potential involvement of CYP1A1 and CYP2B1 in the mutagenic activation of 2-AA by lung S9 was examined in the presence of α-NF, a CYP1A inhibitor, or metyrapone, a CYP2B inhibitor, in the reaction mixtures. Fig. 3 shows that α-NF significantly inhibited the metabolic activation of 2-AA by S9 from saline- and DEP-exposed rats, whereas there was a small but still significant inhibitory effect on S9 from CB-exposed rats. Metyrapone also inhibited the S9-dependent activation of 2-AA mutagenicity from all exposure groups. These results suggest that both the CYP1A1 and CYP2B1 in the lung S9 are capable of activating 2-AA to form mutagenic metabolites.

Fig. 4 shows the direct involvement of CYP1A1 (Fig. 4A) and CYP2B1 (Fig. 4B) in the mutagenic activation of 2-AA, 2-AF, and B[a]P using commercially available CYP supersomes. Fig. 4A demonstrates that CYP1A1 activates 2-AA, 2-AF, and B[a]P. The results show that the sensitivity of different substrates to CYP1A1 supersomes was in the following decreasing order of 2-AF, 2-AA, and B[a]P. Fig. 4B shows that the rat CYP2B1 supersomes containing cytochrome P450 reductase and cytochrome b5 along with glucose-6-dehydrogenase markedly enhanced 2-AF mutagenicity in a dose-dependent manner, but did not convert B[a]P to mutagenic metabolites. Although less pronounced than its effect on 2-AF, CYP2B1 supersomes also increased 2-AA mutagenicity in a dose-dependent manner.

3.3. DEPE mutagenicity

The organic extract of DEP (DEPE) contains diverse compounds including polycyclic aromatic hydrocarbons (PAH), such as B[a]P, and nitro-PAHs, e.g., 1-NP. Fig. 5 shows the direct mutagenicity of DEPE (Fig. 5A) and 1-NP (Fig. 5B). Fig. 5 also shows the enhancing effect of S9 from rats exposed to saline, DEP, and CB at 3 days post-exposure. The results show the direct mutagenicity of 251 ± 23 revertants/plate and 405 ± 6 revertants/plate for DEPE or 1-NP, respectively, in the absence of S9. S9 increased the mutagenicity of DEPE and 1-NP, and DEP or CB exposure did not significantly change this S9-dependent mutagenicity in comparison to the controls. The Aroclor 1254-induced liver S9 was used in this study to compare with the enhancing ability of lung S9 as shown in Fig. 5A and B. The results show that the liver S9 reduced DEPE and 1-NP mutagenicity in a dose-dependent manner, which is in contrast to the activating ability of lung S9.

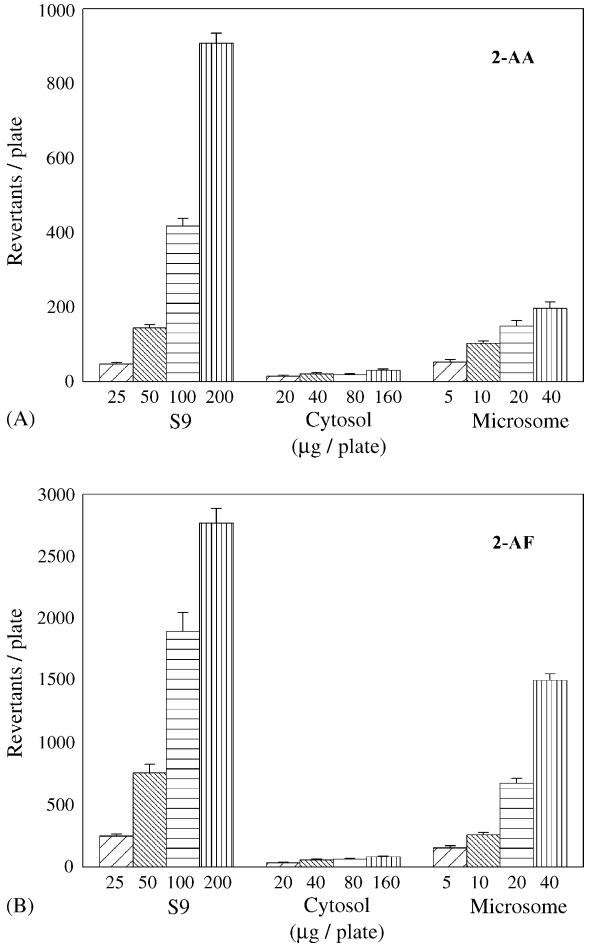

Fig. 2. Effects of lung S9-, cytosolic-, and microsomal-dependent 2-AA (A) and 2-AF (B) mutagenicity in *Salmonella typhimurium* YG1024. S9, cytosol, and microsomes were isolated at 3 days post saline-exposed rat lungs. The activation system was supplemented with glucose 6-phosphate dehydrogenase (1 unit/plate) when isolated microsomes were used. The level of spontaneous revertants was 44 ± 5/plate, 35 ± 2/plate, and 32 ± 2/plate for S9, microsomes and cytosol, respectively. Results are represented as means ± S.E. from three different animals after subtraction of the spontaneous revertants.

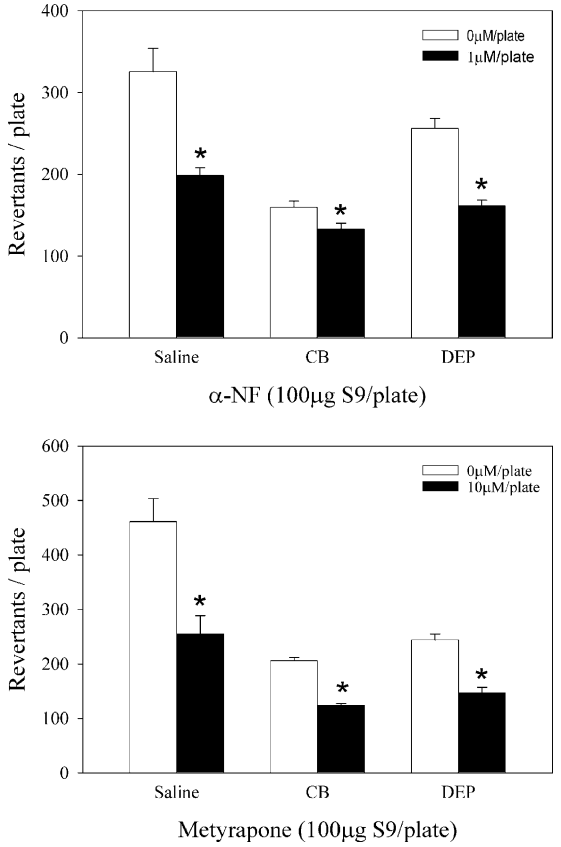

Fig. 3. Effect of CYP1A and CYP2B inhibitors on lung S9-mediated 2-AA mutagenicity. S9 was isolated from rat lungs at 3 days post-exposure to saline, DEP or CB. 2-AA (0.015 μg/plate) mutagenicity was monitored using the Ames test with *Salmonella typhimurium* YG1024 in the absence or presence of α-NF (1 μM/plate), a CYP1A1 inhibitor, or metyrapone (10 μM/plate), a CYP2B1 inhibitor, respectively. Results are represented as mean ± S.E. of the number of revertant colonies from 3 different animals after subtraction of spontaneous revertants (44 ± 5/plate, 35 ± 2/plate, or 32 ± 2/plate for S9, microsomes, and cytosol, respectively). (*) Significantly different from the corresponding control, $p < 0.05$.

The role of CYP1A1 and CYP2B1 in the mutagenic activation of DEPE and 1-NP by lung S9 was demonstrated using CYP supersomes. Fig. 6 demonstrates that CYP2B1 activates DEPE and 1-NP mutagenicity. The results also show that only low concentrations of CYP1A1 supersomes activated DEPE and 1-NP mutagenicity, suggesting that CYP2B1 may play a major role in the activation of the mixed organic components of DEP.

4. Discussion

DEP contain ample carcinogenic compounds that may contribute, at least in part, to the development of lung cancer. However, the mechanisms of DEP-induced lung carcinogenesis have not been fully char-

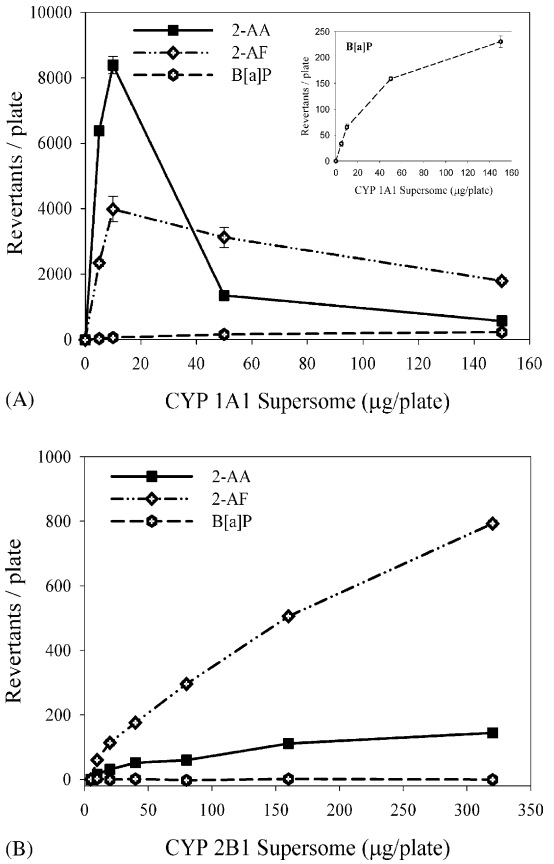

Fig. 4. Dose-dependent effect of CYP1A1 and CYP2B1 on 2-AA, 2-AF, and B[a]P mutagenocity. Rat CYP1A1 (A) or CYP2B1 (B) supersomes, containing P450 reductase in the presence of glucose 6-phosphate dehydrogenase (1 unit/plate), were used as activating enzyme systems. 2-AA (0.015 μg/plate), 2-AF (0.1 μg/plate), and B[a]P (10 μg/plate) mutagenicity were determined by the Ames test with *Salmonella typhimurium* YG1024. CYP1A1-dependent B[a]P activation is shown as the inset. Results are represented as mean ± S.E. of triplicate plates. The level of spontaneous revertants was 38 ± 5/plate and 32 ± 3/plate for CYP1A1 and CYP2B1, respectively.

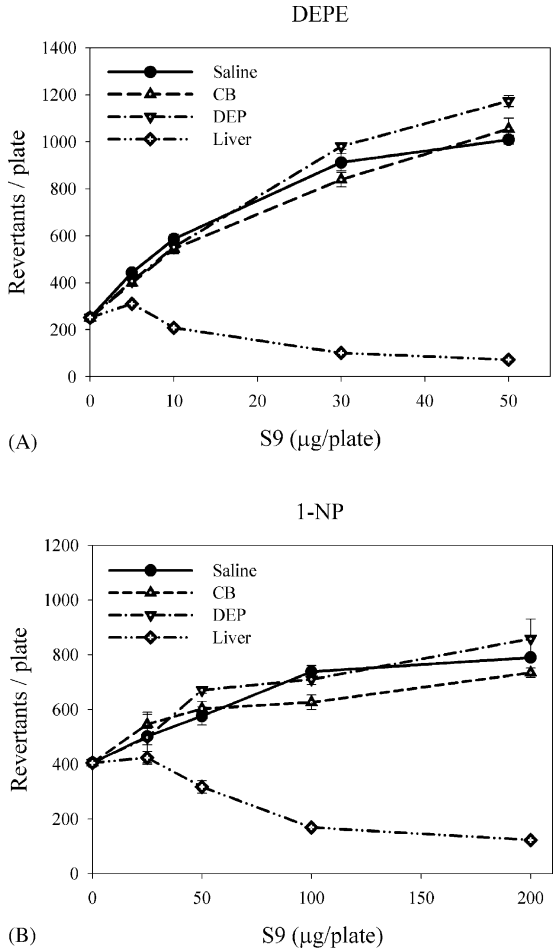

Fig. 5. Protein concentration (μg protein/plate)-dependent effect of lung S9 and liver S9 on DEPE (A) and 1-NP (B) mutagenicity in *Salmonella typhimurium* YG 1024. The lung S9 was isolated from control-, CB-, and DEP-exposed rats at 3 days post-exposure. Aroclor 1254-induced liver S9 was used in parallel as a positive control. The level of spontaneous revertants was 30 ± 5/plate and 38 ± 2/plate for DEPE and 1-NP, respectively. Results are represented as mean ± S.E. of three experiments.

acterized. Exposure of rats to DEP or CB has been shown to significantly alter both phase I and phase II enzymes in the lung, including CYP1A1, CYP2B1, glutathione-*S*-transferase (GST), and NADPH quinone oxidoreductase (QR) [22]. That study shows that DEP-induced alteration of pulmonary P450 enzymes is isoform-selective and time-dependent. The induction of CYP1A1 by DEP, but not CB, was maximal at 1 day post-exposure, but returned to the control levels at 7 days post-exposure. Both DEP and CB significantly decreased CYP2B1 and GST protein levels and enzyme activity up to 7 days post-exposure. In addition, DEP significantly increased QR activity at 7 days post-exposure. Such an alteration of the metabolic enzymes may have a profound effect on the pulmonary bioactivation of pro-carcinogens contained in DEP or other airborne pollutants. The current study was carried out to examine how altered enzyme content, i.e. CYP1A1 and CYP2B1 in particular, may affect the mutagenic-

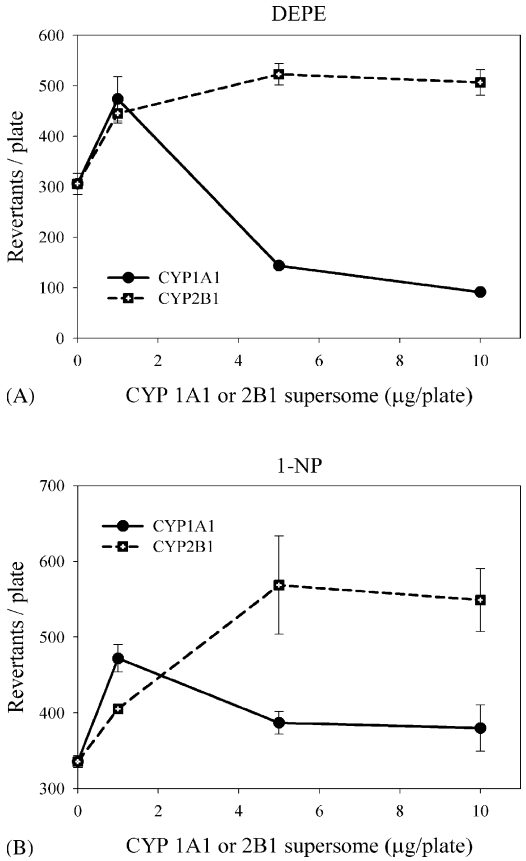

Fig. 6. Dose-dependent effect of CYP1A1 and CYP2B1 supersomes on DEPE (0.01 μg/plate, *panel A*) and 1-NP (0.03 μg/plate, *panel B*) mutagenicity. Rat CYP1A1 or CYP2B1 supersomes, containing P450 reductase in the presence of glucose 6-phosphate dehydrogenase (1 unit/plate), were used as activating enzyme systems. CYP1A1 or CYP2B1-dependent DEPE and 1-NP mutagenicity were determined by the Ames test with *Salmonella typhimurium* YG1024. The level of spontaneous revertants was 38 ± 5/plate and 32 ± 3/plate for CYP1A1 and CYP2B1, respectively. Results are represented as mean ± S.E. of triplicate plates.

ity of known mutagens. The results show that DEP- or CB-exposed lung S9, which contains altered P450 and phase II enzymes, significantly modified the mutagenicity of some known chemicals in the Ames test, while DEP or CB did not affect bioactivation of chemicals associated with DEP.

The induction of 2-AA mutagenicity requires activation by lung S9 in a dose-dependent manner (Fig. 1). At 1 day post-exposure when CYP2B1 was significantly reduced by CB [22], the mutagenicity of 2-AA catalyzed by lung S9 was also reduced by CB exposure. In comparison, DEP which increased CYP1A1 but decreased CYP2B1, depressed the bioactivating potency of S9 on 2-AA mutagenicity at 3 and 7 days post-exposure. This decrease with DEP exposure was less than that for CB exposure, suggesting that both CYP1A1 and CYP2B1 may be involved in the activation of 2-AA mutagenicity. The activation of 2-AA mutagenicity by CYP1A1 has been reported in β-naphthoflavone (a CYP 1A1 inducer)-exposed rat liver and intestine [29]. The present study shows that DEP- or CB-exposed lung S9 reduced 2-AA mutagenicity to below the control level at days 3 and 7 post-exposure, which may be mainly attributed to the reduced lung CYP2B1 content after DEP or CB exposure. Shimada and Guengerich [30] reported that DEP contain not only promutagens, but also compounds which may inhibit metabolic activation of pro-carcinogens. Exposure of rats to DEP or CB may lead to the inhibition of other S9 enzyme systems, such as the FAD monooxygenase capable of *N*-hydroxylating 2-AA and the induction of competing mechanisms resulting in detoxification and/or cofactors depletion. It is possible that DEP- or CB-induced alteration of the phase I and phase II enzymes may increase the detoxification of some active intermediates or shift the metabolism of a substrate from producing mutagenic intermediates to formation of nontoxic metabolites. The results show that CYP2B1 plays an important role in lung S9-dependent 2-AA activation, which is different from the reported role of CPY2B1 from the rat liver. Carriere et al. [29] demonstrated that phenobarbital (a CYP2B1 inducer) treatment significantly reduced the mutagenicity of 2-AA in the liver but not in the intestine, whereas dexamethasone exposure significantly decreased the mutagenicity of 2-AA in both tissues. These studies show that 2-AA appears to be metabolized by various P450 isoforms, which are organ-dependent, to form either mutagenic or non-mutagenic metabolites.

CYP1A1 is present at a very low concentration in the normal lung. However, CYP1A1 is highly inducible by PAHs. Induced CYP1A1 can then metabolize PAHs to ultimate carcinogenic bay-region diol epoxide metabolites [23]. However, CYP2B1 represents the major constitutive P-450 system in the rat lung under basal conditions [24,25]. Alterations of CYP1A1 and CYP2B1 may, therefore, be of great consequence in the pulmonary handling of xenobiotic metabolism. The roles of CYP1A1 and CYP2B1 in mutagenic ac-

tivation of 2-AA in relation to CB or DEP exposure were illustrated using CYP1A and CYP2B inhibitors, α-NF, and metyrapone, respectively. The results show that inhibition of CYP1A1 or CYP2B1 markedly decreased lung S9-mediated 2-AA activation in control as well as CB- or DEP-exposed rats, suggesting that both CYP1A1 and CYP2B1 are involved in the activation of 2-AA mutagenicity by lung S9. To further clarify the roles of CYP1A1 and CYP2B1 in mutagenic activation, rat CYP1A1 and CYP2B1 supersomes were used as activating enzyme systems for 2-AA, 2-AF and B[a]P mutagenicity via the Ames test. The results show that CYP1A1 supersome significantly induced 2-AA, 2-AF and B[a]P mutagenicity. In contrast, increased CYP2B1 supersome concentration markedly increased 2-AA and 2-AF, but not B[a]P, mutagenicity. These results are consistent with the reduction of CYP2B1 by CB or DEP exposure being at least partially responsible for decreased 2-AA and 2-AF mutagenicity, whereas the activation of B[a]P is mainly dependent on the CYP1A1 activation.

The S9 fraction is composed of not only microsomes but also the cytosol, which is known to influence profoundly the mutagenic response of many promutagens [23,31,32]. Since specific forms of cytochrome P-450 have been proposed to activate specific mutagens [33,34], lung S9 activation of 2-AF was also examined to determine substrate specificity in comparison to 2-AA. In the present study, microsomal and cytosolic fractions from lung S9 were used as activating systems to clarify the roles of the cytosol and microsome in the activation of 2-AA and 2-AF mutagenicity. The results show that S9 or microsomes, but not the cytosolic enzymes, activated the mutagenicity of 2-AA and 2-AF in a dose-dependent manner, indicating that the activation of these promutagens by the S9 was partially attributable to microsomal enzymes. However, microsomes did not activate 2-AA or 2-AF to the same level as S9, suggesting that there may be a cooperative mechanism between the microsome and cytosol in S9-mediated 2-AA and 2-AF activation.

The present study demonstrated that DEPE is a direct mutagen and that the mutagenicity of DEPE is further increased with increasing lung S9 concentration. The direct-acting mutagen, 1-NP, has been identified as one of the major genotoxic compounds present in DEPE [15,35] and has been suggested to be used as a marker for the mutagenicity of DEP in workplace atmospheres [15]. El-Bayoumy and Hecht [36] reported the addition of Aroclor 1254-induced liver S9 significantly reduced 1-NP mutagenicity in the Ames test. The current study shows that lung S9 has a dose-dependent inductive effect on the mutagenicity of 1-NP as well as DEPE. Using CYP1A1 or CYP2B1 supersomes as the activating system, the results show that CYP2B1 plays a major role in the lung S9-activated DEPE and 1-NP mutagenicity in a dose-dependent manner. Control studies showed that Aroclor 1254-induced liver S9 reduced the mutagenicity of 1-NP or DEPE as previously reported [36]. The present study shows that enzymes contained in lung S9 are not the same as in the Aroclor 1254-activated liver S9, which reduce the 1-NP mutagenicity.

In summary, the current studies showed that lung S9 was responsible for the activation of promutagens, 2-AA, and 2-AF, and direct mutagens, such as DEPE and 1-NP, in a dose-dependent manner. DEP and CB-mediated changes in pulmonary metabolic activation of mutagenic agents are substrate-specific, and the lung S9 metabolic activity-dependent mutagenicity involves mainly microsomal and, to a lesser extent, cytosolic enzyme activities. There appears to be a cooperative mechanism between microsomal and cytosolic components in lung S9 mediated activation of mutagens. DEP exposure-induced CYP1A1 [22] has been shown to be mainly responsible for PAH metabolism leading to carcinogenic metabolites. However, the results also suggest that the reduction of the lung constitutive isoform CYP2B1, after DEP or CB exposure, may play a role in pulmonary handling of the mutagenic agents.

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