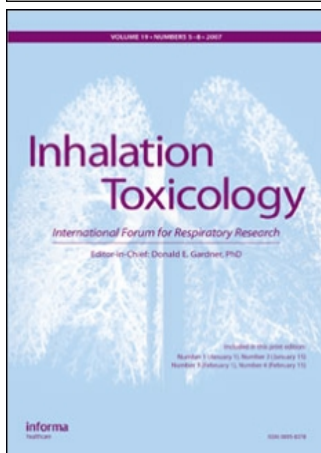


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Antioxidant Enzyme Induction: A New Protective Approach Against the Adverse Effects of Diesel Exhaust Particles

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Exposure to airborne particulate pollutants such as diesel exhaust particles (DEPs) has been associated with allergic respiratory disorders, including asthma and allergic rhinitis. In this communication, we review recent advances in the mechanism by which DEPs elicit their harmful effects and the protective role of antioxidants. Reactive oxidative species (ROS) are believed to play a key role in cellular damage after exposure to DEPs. Numerous reports demonstrate that both proinflammatory and anti-inflammatory products are induced by DEPs via the activation of transcription factors. DEPs trigger multiple signaling pathways, which lead to DNA damage and cell apoptosis, inflammatory response, and antioxidant defense. Recent studies both in vitro and in mice show that antioxidants could alleviate the allergic inflammatory effects of DEPs. Human in vivo models suggest that the important phase II enzymes GSTM1 and GSTP1 modify the adjuvant effect of diesel exhaust particles on allergic inflammation. We have shown that the induction of phase II enzymes by the chemical sulforaphane can block DEP-induced enhanced immunoglobulin (Ig) E production in B cells and DEP-induced proinflammatory cytokine production in epithelial cells. These findings suggest that overexpression of antioxidant enzymes could constitute a powerful potential chemopreventive approach against adverse effects induced by oxidant pollutants such as DEPs.

INTRODUCTION

The prevalence rate of allergic diseases such as bronchial asthma and rhinitis is increasing in most industrialized countries of the world. The growing epidemiological and experimental evidence demonstrates that exposure to oxidant pollutants such as diesel exhaust particles (DEPs) is one of the multiple factors that may be responsible for this increase (Diaz-Sanchez et al., 2003). Because DEPs are a major component of ambient fine particulate matter (PM_{2.5}), especially in urban areas, several studies have sought to clarify the association between DEPs and allergic disorders (Diaz-Sanchez et al., 1994; 1997; 1999). These studies have led to the concept that the key pathway in the mode of action of DEPs is the generation of reactive oxygen species (ROS) that lead to a state of cellular oxidative stress, which in turn results in significant damage to cell structures (Hiura et al., 1999; Li et al., 2002a; Kumagai et al., 1997). In vitro studies show that DEPs upregulate both proinflammatory

mediators and antioxidant enzymes in multiple target cells such as bronchial epithelial cells, monocytes, and lymphocytes (Al-Humadi et al., 2002; Takizawa et al., 1999; Bayram et al., 1998; Tsien et al., 1997; Takizawa et al., 2000). These cellular studies suggest that exogenous antioxidants may mitigate the adverse effects of DEPs including DNA damage, cell apoptosis and inflammation responses (Han et al., 2001; Hiura et al., 1999; Blanchet et al., 2004; Shima et al., 2006). In addition, we have shown that endogenous antioxidants may also act to counteract the adverse effects of oxidant pollutants on allergic airway responses. We have shown that in a human in vivo model the phase II enzymes glutathione *S*-transferase superfamily GSTM1 and GSTP1 can modify the adjuvant effects of DEPs on allergic inflammation (Gilliland et al., 2004). This communication discusses what is known about the potential mechanism of action of DEP and show how we can use this information to devise a possible strategy for chemoprevention.

CHARACTERIZATION OF DIESEL EXHAUST PARTICLES

Diesel exhaust contains small particles that range from nanoparticles to coarse particles, with mass concentrated in the accumulation mode centered at 0.2 μm in diameter. These particles have high deposition rates in the lung and can remain for

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lengthy periods in the atmosphere. However, these primary particles will rapidly coalesce to form chains and aggregates of a broad range of sizes, and are important contributors to both particulate matter less than 10 μm in diameter (PM₁₀) and PM_{2.5} in ambient air. Whether the main biological impact of DEP is through primary particles or through their aggregates has yet to be determined. DEPs consist of a carbonaceous core with a large surface area to which chemicals are absorbed. These include organic chemicals such as polycyclic aromatic hydrocarbons (PAHs), nitro derivatives of PAHs, oxygenated derivatives of PAHs (ketones, quinones, and diones), heterocyclic compounds, aldehydes, and aliphatic hydrocarbons. The PAHs and their oxygenated derivatives (e.g., quinones) have attracted special attention because they are able to redox cycle and generate reactive oxygen species (ROS) in target cells.

REACTIVE OXYGEN SPECIES GENERATION IN VIVO AND IN VITRO AFTER EXPOSURE TO DEPS

Reactive oxygen species (ROS) include oxygen ions, free radicals, and peroxides, both inorganic and organic. Intracellular ROS production is believed to participate in cell activation after exposure to diesel exhaust particles. Production of superoxide (O_2^-) and hydroxyl radical (OH) from organic extracts of DEP was reported for in vitro studies (Kumagai et al., 1995; Sagai et al., 1993). Inhaled or intratracheally instilled DEP also generate ROS in the lungs of exposed mice, and this ROS can cause protein oxidation, lipid peroxidation, and DNA damage (Donaldson et al., 2003; Martin et al., 1998; Nel et al., 1998; Sagai et al., 1993). In addition, direct observation of OH generation in the lungs of living mice after intratracheal instillation of DEP has been seen using electron spin resonance spectroscopy (Han et al., 2001).

While it is still not completely determined how ROS are generated by DEPs, it is known that two main families of organic compounds are adsorbed on DEP: PAHs and quinones. PAHs could be desorbed from DEP and become available to bind to the cytosolic aryl hydrocarbon receptor and induce gene expression-like phase I metabolism enzymes, especially cytochrome P-450 1A1 (CYP1A1) (Bonvallot et al., 2001; Rengasamy et al., 2003; Takano et al., 2002). PAH metabolism produces electrophilic and reactive metabolites, including ROS. A role for PAH is supported by the correlation between the PAH content of fine and ultrafine particles and their ability to induce oxidative stress in macrophages (Li et al., 2002b). Quinones are known to generate oxidative stress by redox cycling. They are suspected to be responsible for the production of O_2^- and OH radicals detected in methanol extracts of DEP. Redox cycling quinones undergo one-electron reductions by NADPH cytochrome P-450 reductase to form semiquinones (Monks et al., 1992). These semiquinones can be recycled to the original quinones, leading to the formation of O_2^- . The detoxification of quinones can occur by two-electron reduction performed, among others, by the phase II enzyme NADPH-quinone

oxidoreductase 1 (NQO-1). In addition to participating in ROS production, quinones are electrophiles that can induce covalent modification of proteins and DNA strands (Monks et al., 1992). This can lead to irreversible damage in tissues. Metals present on diesel particles may also generate ROS. Aust et al. (2002) postulated that bioavailable transition metals from inhaled airborne particulates could catalyze redox reactions in human lung epithelial cells. They showed that phosphate buffer-soluble fractions of diesel particles could generate ROS and that this activity was inhibited over 90% by a metal chelator. Valavanidis et al. (2005) used electron paramagnetic resonance to show that diesel particles can generate hydroxyl radicals and that this was related to soluble iron ions.

It should also be appreciated that inflammation per se is an oxidative event (Bowler and Crapo, 2002). Inflammatory cells themselves are an important source of ROS. Thus, particles devoid of PAH/quinones and metals can also induce oxidative stress and ROS in vivo at high concentrations. Diesel particles may thus increase oxidative stress directly via the induction of ROS by the chemicals they contain and/or indirectly by causing enhanced inflammation and thus additional ROS generation.

To maintain cellular redox equilibrium, the potentially injurious effects of ROS and oxygen radicals are neutralized by a variety of antioxidants including glutathione, superoxide dismutase (SOD), and NQO1. Under conditions of abundant ROS production, the antioxidant defenses may be overwhelmed, leading to a state of cellular oxidative stress. Oxidative stress is believed to be the underlying mechanism of DNA damage such as DNA adducts and DNA strand breakages, and of cell apoptosis and proinflammatory cytokines release in cells exposed to DEPs.

EVIDENCE OF OXIDATIVE DNA DAMAGE VIA ROS GENERATED BY DEPS

In recent years, generation of reactive oxygen species capable of inducing cellular oxidative stress has been the main focus of researchers studying the underlying mechanisms behind the genotoxic effects of particles. ROS possess high reactivity with DNA, proteins, and lipids. The most abundant and mutagenic ROS-induced DNA lesion, 8-hydroxydeoxyguanosine (8-OH-Gua), causes G:C to T:A transversions and thus could be responsible for mutations that lead to carcinogenesis. 8-OH-Gua was found to be increased in the mouse lung DNA after the administration of DEP (Ichinose et al., 1997; Nagashima et al., 1995; Risom et al., 2003). Tsurudome et al. showed that 8-OH-Gua levels in rat lung DNA increase markedly at an early phase after DEP exposure (Tsurudome et al., 1999). DEP induced a significant increase in DNA strand breaks in the colon and liver of rats exposed via the gastrointestinal route (Dybdahl et al., 2003). Following DEP exposure, mRNA expression of OGG1 (8-oxoguanine DNA glycosylase, an enzyme involved in repair of 8-OH-Gua) is elevated in liver (Dybdahl et al., 2003) or lung (Risom et al., 2003; Tsurudome et al., 1999), and ERCC1 (excision-repair cross-complementary 1), an enzyme

involved in the nucleotide excision pathway, is also increased in the liver (Dybdahl et al., 2003).

TRANSCRIPTION FACTORS AND SIGNALING PATHWAYS TRIGGERED BY DEPs

DEPs induce a dose-dependent activation of nuclear factor- κ B in human bronchial epithelial cells (BECs), (Takizawa et al., 1999; Bonvallot et al., 2001). Ma et al. demonstrated that exposure to DEP at a non-cytotoxic concentration significantly enhanced the transactivation of NF- κ B, but not activator protein-1 (AP-1) in mouse epidermal cells (Ma et al., 2004). Zhang et al. showed DEPs markedly upregulated expression of fra-1, a heterodimeric partner of AP-1, in a murine lung epithelial cells (Zhang et al., 2004). Overexpression of fra-1 downregulated c-Jun, and Nrf2 enhanced AP-1 and antioxidant response element-mediated reporter gene expression, respectively. Furthermore, inhibitors of ERK1/2, JNK1, and p38 mitogen-activated protein kinases (MAPKs) significantly suppressed DEP-stimulated fra-1 transcription, suggesting their involvement in the induction process. These findings suggest that fra-1 induction by DEP may play a role in selectively regulating gene expression involved in alveolar epithelial cell injury and repair. Li et al. showed that induction of the antioxidant enzyme heme oxygenase-1 (HO-1) was mediated through accumulation of the bZIP transcription factor (Nrf2) in the nucleus and that Nrf2 gene targeting significantly weakens this response in human epithelial cells and mouse lung macrophage cells (Li et al., 2004). This pathway is sensitive to pro-oxidative and electrophilic DEP chemicals and is also activated by ambient ultrafine particles.

DEP chemicals also induced the activation of stress-activated protein kinases, including p38 MAP kinase and Jun kinase, which play a role in cellular apoptotic pathways (Hiura et al., 1999; Hashimoto et al., 2000; Li et al., 2004). The production of interleukin-8 and RANTES (regulated on activation, normal T-cell expressed and secreted) was blocked by SB203580, an inhibitor of p38 MAP kinase. Intercellular adhesion molecule (ICAM) induction by DEPs was also inhibited by SB203580, but not by the protein kinase C (PKC) inhibitor staurosporin or PD 98059 (mitogen-activated protein kinase kinase-1 inhibitor). The DEP-induced cell number increase was dose-dependently inhibited by the N-terminal c-jun kinase inhibitor SP600125 in human lung epithelial cell. Li et al. showed that c-Jun N-terminal kinase activity increased in DEP-stimulated human BECs and macrophages (Li et al., 2002b).

DEP promoted phosphorylation of Akt, a substrate of phosphatidylinositol 3-kinase (PI3K), on Ser-473 and Thr-308 in a PI3K-dependent manner, and enhanced phosphorylation of downstream p70/p85 S6 kinases (p70/p85S6K) as well as glycogen synthase kinase-3 β (GSK-3 β) (Ma et al., 2004). Blockage of PI3K activation eliminated DEP-stimulated NF- κ B transactivation. This suggests that DEP-induced transactivation of NF- κ B is mediated by the PI3K/Akt signaling pathway. Kaimul

Ahsan et al. showed that the downregulation of Akt phosphorylation by DEP resulted in apoptosis (Kaimul Ahsan et al., 2005).

EVIDENCE OF PROTECTIVE ROLE OF ANTI-OXIDANT PROTEIN IN THE HARMFUL EFFECTS CAUSED OR EXACERBATED BY DEPS

Given the studies just reviewed, it seems clear that generation of ROS plays an important role in the mechanism of DEP's action. Consequently, we believe that the ability to form an appropriate endogenous antioxidant response will determine the severity of proinflammatory and adjuvant responses caused by DEP and similar pollutants. We propose that Nrf2-mediated phase II enzyme expression protects against the proinflammatory effects of particulate pollutants in the setting of allergic inflammation and asthma.

Glutathione S-transferase is an enzyme that is involved in the detoxification of environmental chemicals, including redox-cycling components in tobacco smoke and PM. There is accumulating evidence that GSTP1 and GSTM1 could be associated with susceptibility to allergic response and asthma caused or exacerbated by airborne particulates. We have shown the importance of genetic polymorphisms in the expression of GST enzymes in allergic responses after nasal exposure to diesel particles (Gilliland et al., 2004). Patients sensitive to ragweed allergen were challenged intranasally with allergen alone and with allergen plus DEPs. In this single-blind, randomized, placebo-controlled study, patients with genetic polymorphisms that resulted in the absence or diminished production of GSTM1 or GSTP1 respectively had a significantly larger increase in IgE and histamine in comparison to patients with a functional GSTM1 and GSTP1 genotype. These findings suggest GSTM1 and GSTP1 modify the effect of diesel exhaust particles on allergic inflammation. GSTM1 and GSTP1 have also been demonstrated to have a protective role in protecting against asthma in population-based studies. In an evaluation of over 200 people in northern Europe, bronchial hyperresponsiveness, a key phenotype of asthma, was associated with a GSTP1 polymorphism (Fryer et al., 2000). The people carrying Val105/Val105 reported has a sixfold lower risk of asthma. A case-control study indicated that polymorphisms at the GSTP1 might account for the variations in response to inhaled particles (Spiteri et al., 2000). The frequency of the GSTP1 Val/Val genotype was reduced in atopic individuals. Strange et al. have drawn similar conclusions that the GSTP1 Val105/Val105 was essentially protective against asthma (Strange et al., 2001). Based on these findings, we speculate that genetic polymorphisms in antioxidant defense genes play a role in the susceptibility to adverse effects and asthma caused or exacerbated by airborne particulates.

FUTURE DIRECTIONS: POSSIBLE STRATEGY FOR CHEMOPREVENTION

Exogenous antioxidants reduce the allergic inflammation effects of DEPs in vitro and in mice (Whitekus et al., 2002).

However, early trials in our laboratory using inhaled *N*-acetylcysteine (Mucomist) to block the effects of DEP in our human nasal in vivo model proved ineffective. The reasons for this are probably poor bioavailability and the inability of the antioxidant to penetrate into the relevant cells at a suitable dose. Given our results on GSTM1 and GSTP1 genes, we hypothesized that induction of endogenous antioxidants such as phase II enzymes may present a better preventive strategy against adverse health effects of DEPs. In recent studies conducted in our lab, we studied the association of phase II antioxidant enzymes and IgE production increased by DEPs and its extracts (DEPX) (Wan & Diaz-Sanchez, 2006). We investigated the regulation in B cells of NQO1 as a typical model phase II enzyme and its role in modulating DEPX-enhanced IgE responses. DEPX increased NQO1 mRNA expression in both peripheral blood lymphocytes and the B lymphocyte cell line in a dose-dependent manner. Phosphatidylinositol 3-kinase (PI3-K) and p38 MAP kinase participated in induction of both NQO1 mRNA and protein expression. The expression levels of phase II enzymes including NQO1, GSTP1 and GSTM1 were increased through the addition of sulforaphane, a potent inducer of phase II enzymes. The ability of DEPX to enhance IgE production was blocked by induction of phase II enzymes in B cells by the chemical sulforaphane. These findings suggest that a natural protective mechanism in B cells from oxidant pollutants such as diesel particles is the expression of phase II enzymes through induction of antioxidant responsive elements.

Similarly, we have shown that sulforaphane can also increase phase II enzyme production and GST activity in human bronchial epithelial cells and that this can result in inhibition of DEPX-induced promotion of proinflammatory cytokines such as IL-8 (Ritz et al., 2007). The ability of sulforaphane to act on at least two cell types to mitigate the effects of DEP chemicals is encouraging. These studies support the approach of overexpression of phase II as a potential future chemopreventative strategy to decrease the proinflammatory and proallergic effects of DEPs. Given that other adverse effects of DEPs (such as oxidative DNA damage) are thought to be mediated by similar pathways, it is tempting to speculate that this strategy may have a broad application. The effectiveness of this approach needs yet to be tested with in vivo models.

SUMMARY

The accumulating evidence suggests the DEPs are associated with the allergic diseases and asthma. The underlying mechanism of the cellular damage caused by DEPs seems to be the generation of ROS, which trigger multiple transcription factors and signaling pathways. Endogenous antioxidant enzymes can protect against allergic inflammation both in vivo and in vitro. Accordingly, identifying these potential protective factors is an important step in developing and testing interventions to reduce the harmful effects of oxidant pollutants

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