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Chapter 16

ANTIOXIDANT INTERVENTIONS AS POTENTIAL PROTECTIVE STRATEGIES FOR POPULATIONS SUSCEPTIBLE TO DIESEL EXHAUST PARTICLES

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ABSTRACT

$PM_{2.5}$-induced oxidative stress is intimately associated with the progression and exacerbation of many diseases, such as allergic respiratory and ischemic cardiovascular diseases. Diesel exhaust particles (DEP) are a major component of fine ambient particulate matter with an aerodynamic diameter of <2.5 μm ($PM_{2.5}$). Studies have shown that most of the effects of DEP are due to reactive oxygen species (ROS) generated directly and indirectly by exposure to DEP within exposed cells, such as epithelial cells, macrophages, and endothelial cells.

Antioxidants/redox-modulating agents such as N-acetyl-L-cysteine (NAC) have been reported to modulate various cellular and biochemical responses caused by DEP exposure. Endogenous antioxidants such as sulforaphane is the most potent inducer of phase II enzymes identified to date and is thought to act via activation of the Nuclear factor-like 2 (Nrf2) transcription factor. Gene polymorphisms involved in antioxidant pathways can modify responses to air pollution exposure. Therefore, it will be necessary to identify susceptible populations in order to perform targeted prevention of DEP-induced oxidative stress.

This review discusses the targeting of systemic and local oxidative stress with antioxidants/redox-modulating agents or boosting the endogenous levels of antioxidants in order potentially to provide new chemoprevention strategies to counteract oxidative stress induced by DEP in susceptible populations.

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Keywords: particulate matter, reactive oxygen species, antioxidants/redox, Nrf2

INTRODUCTION

Epidemiological studies have shown that $PM_{2.5}$ is associated with increased respiratory morbidity and mortality [1-3]. Especially in urban areas, many studies have been performed to clarify the association between $PM_{2.5}$ and disorders such as asthma [4, 5], ischemic cardiovascular diseases [6, 7], arteriosclerosis [8, 9], cancer [10, 11], and neurological disorders [12, 13].

Diesel exhaust (DE) particles (DEP) are the major component of ambient PM2.5 [14]. *In vitro* studies have shown that most of the effects of DEP are due to reactive oxygen species (ROS) generated by exposure to DEP and the subsequent generation of the oxidative stress response within exposed cells [15-22]. Strong experimental evidence supports the relationship between DEP-induced oxidative stress and the exacerbation of these related environmental diseases *in vivo* [23-34]. A recent human crossover study reported that systemic miRNA with plausible biological function is altered by acute moderate-dose DEP exposure, and oxidative stress appears to mediate DEP-associated changes in miR-144 [35].

Repeated low-level ($100\mu g/m^3$) DEP exposure in two different strains of mice induced differential susceptibility to DEP exposure [23, 24, 28]. Gene polymorphisms involved in antioxidant pathways can modify responses to DEP exposure [36]. Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) is a redox-sensitive basic leucine zipper transcription factor involved in the transcriptional regulation of many antioxidant genes [37]. It is a key regulator of antioxidant defense and constitutes the main defense against the proinflammatory and oxidizing effects of DEP *in vitro* [21] and *in vivo* [25]. Thus, an imbalance between DEP-induced oxidative stresses versus host antioxidant responses may be key for the development of hazardous health effects.

It is important to protect populations susceptible to such pollution. While the characterization and monitoring of pollutant components currently dictates pollution control policies, it will be necessary to identify susceptible populations in order to perform the targeted prevention of pollution effects, such as DEP-induced oxidative stress. Targeting systemic and local oxidative stress with antioxidants/redox-modulating agents or boosting the endogenous levels of antioxidants in order to maintain the balance between oxidative stresses versus antioxidants may provide new chemoprevention strategies to counteract oxidative stress caused by DEP. This review will describe recent advances in our understanding of possible chemoprevention strategies for populations susceptible to oxidative stress caused by DEP.

DEP-INDUCED OXIDATIVE STRESS

Diesel exhaust contains small particles that range in size from nanoparticles to coarse particles with an accumulation mass mode of 0.2 μm in diameter. Particles of this size have high deposition rates in the lung, and can persist in the atmosphere for lengthy periods [38].

These primary DEP coalesce to form aggregates of a broad range of sizes and are important contributors to $PM_{2.5}$ in ambient air. DEP consist of a carbonaceous core with a large surface area, to which organic compounds such as polycyclic aromatic hydrocarbons (PAH) are adsorbed. PAH and their oxygenated derivatives (e.g., quinones) have attracted particular attention because they are able to affect the redox cycle and generate ROS in target cells [39-41]. Studies of molecular mechanisms have focused on the role of ROS generated directly and indirectly by exposure to DEP both *in vitro* [15-22] and *in vivo* [23-34].

BALANCE OF OXIDANTS/ANTIOXIDANTS

The susceptibility to air pollution is determined by gene-environmental interaction. Host responses to oxidative stress induced by DEP are regulated by a balance between antioxidants and proinflammatory responses. ROS generated by DEP are responsible for protein oxidation, lipid peroxidation, and DNA damage in target cells, such as macrophages and epithelial cells [20, 21]. Additional attempts to defend against oxidative tissue damage lead to a depletion of cellular glutathione reserves and a drop in the cellular reduced glutathione (GSH)/glutathione disulfide (GSSG) ratio. This state of oxidative stress incites further cellular antioxidant responses, such as the induction of heme oxygenase 1 (HO-1) expression, the production of proinflammatory cytokines, and cellular apoptosis [42]. Nrf2 is a key transcription factor that regulates antioxidant defense in macrophages and epithelial cells that act as the main defense against the proinflammatory and oxidizing effects of DEP [21]. Xiao et al. reported [19] that cytoprotective pathways are induced by the Nrf2 transcription signal pathway at the lowest levels of oxidative stress, which may constitute the first tier of a hierarchical oxidative stress response, as in the production of HO-1, among others. If these enzymes fail to neutralize the effects of ROS, proinflammatory effects constitute a second tier or superimposed level of oxidative stress. The final tier or superimposed level of oxidative stress is cytotoxicity, including the initiation of programmed cell death. These responses are dependent on the activation of the mitogen-activated protein kinase (MAPK) and NF-kB signaling cascades as well as activation of the antioxidant response element [19, 42].

Extensive studies have suggested that Nrf2 contributes to protection against various pathologies, including asthma [43], chronic obstructive pulmonary disease (COPD) [44], idiopathic pulmonary fibrosis (IPF) [45], atherosclerosis [46], ischemic cardiovascular diseases [47], heart failure [48], carcinogenesis [49], Alzheimer's disease [50], autism [51], influenza infection [52], and inflammatory disorders [53]. It is considered that the populations that have these diseases are susceptible to oxidative stress caused by DEP. Indeed, experimental evidence supports a relationship between DEP and the exacerbation of these diseases [25-34, 54, 55]. Oxidative stress seems to be involved in DEP-associated increases in airway inflammation [25], allergic asthma [26], lung fibrosis [27], and oxidative DNA damage [30], as evidenced by experiments in *Nrf2* knockout mice.

Gene polymorphisms involved in antioxidant pathways can modify responses to DEP exposure. Gilliland et al. [36] reported that individuals with *glutathione S-transferase* (*GST)M1* null or the *GSTP1* I105 wild type genotypes showed enhanced nasal allergic responses in the presence of DEP, and the DEP enhancement was largest in patients with both the *GSTM1* null and *GSTP1* I/I genotypes.

This strongly suggests that antioxidants may represent an effective prophylactic strategy against the adverse health effects of DEP among susceptible populations. Therefore, gene-related host susceptibility to air pollutants should be considered when a large-scale cohort study is undertaken.

ANTIOXIDANT PROTECTIVE STRATEGIES FOR DEP-INDUCED HEALTH EFFECTS

Many studies have indicated that antioxidants can exhibit protective health effects against DEP-induced oxidative stress (Table 1). We summarize the overall potential protective role of antioxidants represented by the Nrf2 and NF-κB activation signal pathway against DEP in Figure 1. Targeting systemic and local oxidative stress with antioxidants/redox-modulating agents or boosting the endogenous levels of antioxidants may provide new chemoprevention strategies to counteract the ROS induced by DEP for susceptible populations.

Antioxidants/Redox-Modulating Agents

Thiol Antioxidant

N-acetyl-L-cysteine (NAC) is an acetyl derivative of the amino acid cysteine and is a strong reducing agent. NAC is deacetylated into cysteine and also serves as a precursor of GSH in cells. Its ability to reduce disulfide bonds makes it a good reducing agent and also allows it to neutralize oxidant species [56]. *In vitro* studies have shown that DEP-induced ICAM-1, IL-8, eotaxin, and RANTES expression is inhibited by NAC in human bronchial epithelial cells (HBEC) [15-18]. Indeed, DEP-induced NF-κB activation was completely inhibited by pretreatment with NAC [16]. DEP-induced activation of MAPK pathways was also blocked by NAC [18]. DEP induce a dose-dependent decrease in the GSH/GSSG ratio in macrophage cells, in parallel with a linear increase in the number of newly expressed proteins. More than half of these proteins were found to be suppressed in the presence of NAC. These include antioxidant enzymes, for example, HO-1 and catalase, as well as proteins that play a role in pulmonary inflammation [19]. NAC also decreases antioxidant enzymes induced by DEP in endothelial cells [57]. *In vivo* studies have shown that repeated low-level DEP exposure in two different strains of mice induced differential susceptibility to DEP exposure and NAC decreased DEP-induced airway proinflammatory responses in highly reactive mice [23]. In an ovalbumin (OVA)-induced allergic murine model, NAC treatment reduced the increases in peribronchial eosinophils and mucous goblet cells seen in lung tissues, and the increases in Th2 cytokines seen in BAL fluid, after DEP exposure [28]. Yamamoto et al. [35] recently reported that alterations in systemic miRNA profiles were associated with acute DE exposure in humans as well as oxidative stress, using controlled exposure and a randomized crossover design. Interestingly, miR-144 was up-regulated by DE and was associated with systemic oxidative stress; the DE-related increase in miR-144 was prevented by NAC supplementation. This study also indicated that increases in miR-144 and miR-21 were associated with plasma 8-hydroxydeoxyguanosine (8-OHdG) level and were blunted by NAC. Because the formation of 8-OHdG is an important factor in the initiation of DEP-induced

lung carcinogenesis in mice [58], it is considered that NAC may be able to counteract DEP-induced lung carcinogenesis.

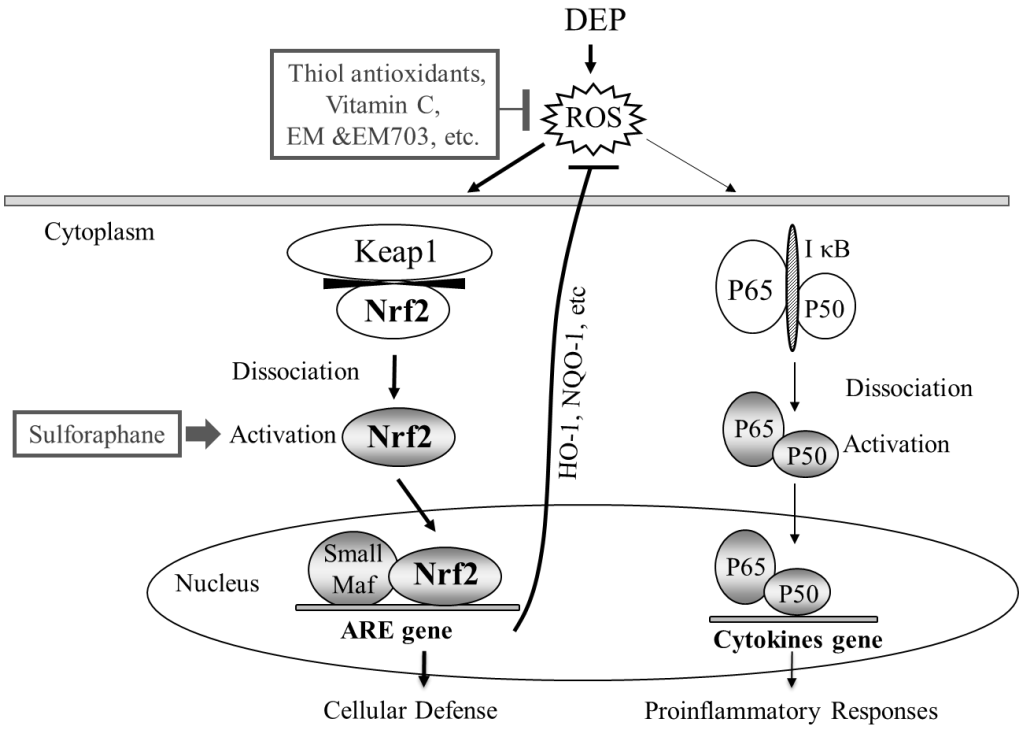

Figure 1. Schematic of the potential protective role of antioxidant signal pathway against DEP *in vitro*. Nrf2 plays an important role in cellular defense mechanisms against oxidant stresses induced by DEP. Under normal conditions, Nrf2 is sequestered in the cytoplasm via binding to its repressor molecule, Kelch-like ECH-associated protein 1 (Keap1). ROS induced by DEP cause dissociation of the Nrf2-Keap1 complex, which activates the nuclear translocation of Nrf2. After heterodimerization with other transcription factors such as small Maf, Nrf2 promotes the transcriptional activation of antioxidants and detoxifying enzymes by binding to the antioxidant responsive elements (ARE) in the promoter regions of the target genes. Simultaneously, via phosphorylation of the inhibitor molecule IκB, oxidative stress causes activation of NF-κB, leading to transcriptional activation of genes encoding proinflammatory mediators such as cytokines. Antioxidants/redox-modulating agents such as thiol antioxidants, vitamin C, EM, and EM703 reduce DEP-induced ROS production. Boosting the endogenous levels of antioxidants such as sulforaphane increases the expression of phase II enzymes such as HO-1 via activation of the transcription factor Nrf2. These antioxidants result in the reduction of proinflammatory responses by redox regulation of the activation of NF-κB signaling.

Bucillamine (BUC), a cysteine derivative that contains two donatable thiol groups, is capable of replenishing the thiol group in glutathione, thereby reactivating this endogenous defense against oxidant injury [59]. Whitekus et al. [42] reported that NAC and BUC effectively prevented the enhancement of OVA-specific IgE and IgG1 production in animals co-challenged by DEP inhalation. Moreover, the same agents also decreased the generation of lipid peroxides and carbonyl proteins in the lungs of OVA plus DEP-exposed animals.

Thioredoxin-1 (Trx-1) is a multifunctional redox (reduction/oxidation)-active protein that scavenges reactive oxygen species by itself or together with Trx-dependent peroxiredoxin. Kaimul Ahsan et al. [60] reported that Trx-1 scavenged ROS generated by DEP and

attenuated lung injury using human Trx-1 (hTrx-1)-transgenic mice *in vivo*, and DEP-induced ROS generation was suppressed by hTrx-1 transfection or pretreatment with recombinant human (rh) Trx-1 *in vitro*. An in vivo study also reported that the administration of rhTrx-1 attenuated inflammatory cytokines or lung injury in mice [61]. Trx-1 may thus have potential as a clinical therapeutic agent against DEP-induced acute lung injury and cellular damage.

Procysteine (cysteine l-2-oxothiazolidine-4-carboxylic acid, OTC) is a cysteine-relieving compound that increases the intracellular cysteine levels, and has greater bioavailability than NAC. This thiol compound is well tolerated and has been shown to increase mitochondrial levels of GSH in alveolar type II cells [62]. Nemmar et al. [29] reported that pulmonary exposure to DEP causes oxidative stress that is responsible, at least in part, for the pulmonary and systemic inflammation and thrombotic events in the pial cerebral microvessels of mice. OTC pretreatment abrogated these effects through its ability to balance oxidant-antioxidant status.

Vitamin

Vitamin C is the major water-soluble antioxidant and acts as the first defense against free radicals in whole blood and plasma [63]. It has been reported that vitamin C partly attenuated both DEP-generated oxidative stress and the expression of cell surface adhesion molecules in human umbilical vein endothelial cells (HUVEC) [64]. Since oxidative DNA damage plays an important role in the carcinogenesis of DEP [58, 65], Møller et al. [66] investigated genotoxic and oxidative stress effects of DEP in guinea pigs, while monitoring the ascorbate status. The results indicate that oxidative DNA damage is formed independently of the vitamin C status. In contrast, reduced vitamin C intake in guinea pigs increased the oxidation of protein and lipid in the liver [67]. Thus, it is possible that vitamin C depletion could enhance the susceptibility to DEP in guinea pigs, as shown for lipid peroxidation. Future studies should address whether additional depletion of other antioxidant compounds is required for oxidative DNA damage to occur following exposure to particulate matter.

Macrolides

Erythromycin (EM) is a 14-membered-ring macrolide (14-MRML) that has been reported to improve the survival of patients with diffuse panbronchiolitis (DPB) by several anti-inflammatory mechanisms [68]. EM703 is a new 12-membered-ring macrolide derivative of erythromycin without antibacterial effects [69]. It has been reported that not only EM but also EM703 has the anti-inflammatory action of a macrolide, which is independent of its antibacterial activity [70, 71]. Moreover, it is known that the superoxide scavenging activity of erythromycin-iron complex contributes to the anti-inflammatory action of EM [72] and the action of anti-oxidative stress of EM has therapeutic value for influenza-virus-induced pneumonia [73]. In terms of the anti-inflammatory effects of EM and EM703 on the expression of IL-8 caused by DEP exposure in HBEC, the results suggested that both EM and EM703 are promising anti-inflammation and antioxidant drugs, particularly EM703, which lacks antibacterial effects [74]. EM and EM703 may contribute to chemical prevention of the risk of pulmonary diseases induced by oxidative stress from environmental pollutants via anti-inflammatory effects and anti-oxidative stress, such as DEP.

Table 1. The efficacy of antioxidants in DEP-induced oxidative stress

Antioxidants[*1]			Study Aim[*2]	Outcome	References
Antioxidants/ redox-modulating agents	Thiol antioxidant	NAC	Effect of NAC on proinflammatory mediators in HBEC	Decrease proinflammatory mediators induced by DEP with NAC	[15-18]
		NAC	Effect of NAC on hierarchy of oxidative stress effects in macrophages	Decrease hierarchy of oxidative stress induced by DEP with NAC	[19]
		NAC	Effect of NAC on proinflammatory mediators in endothelial cells	Decrease proinflammatory mediators induced by DEP with NAC	[57]
		NAC	Effect of NAC on inflammatory cytokines in mice	Decrease inflammatory cytokines induced by DEP with NAC group	[23]
		NAC	Effect of NAC on Th2 cytokines in OVA allergic airway inflammation in mice	Decrease Th2 cytokines induced by DEP with NAC group	[28]
		NAC	Effect of NAC on the OVA-specific IgE and IgG1 production in mice	NAC effectively prevented the enhancement of OVA-specific IgE and IgG1 production by DEP inhalation	[42]
		NAC	Effect of NAC on peripheral blood cellular miRNA in human asthma	Oxidative stress appears to mediate DE-associated changes in miR-144. Increases in miR-144 were blunted by NAC	[35]
		BUC	Effect of BUC on the OVA-specific IgE and IgG1 production in mice	BUC effectively prevented the enhancement of OVA-specific IgE and IgG1 production by DEP inhalation.	[42]
		Trx	Effect of Trx on scavenging ROS and lung injury in mice	Plays a role in protection against DEP-induced lung damage by regulating Akt-mediated antiapoptotic signaling	[60]
		OTC	Effect of OTC on lung inflammation and cerebral microvessel thrombosis in mice	OTC abrogated lung inflammation and cerebral microvessel thrombosis promoted by DEP	[29]
	Vitamin	Vitamin C	Effect of vitamin C on expression of cell surface adhesion molecules in HUVEC	Vitamin C partly attenuated DEP-generated expression of cell surface adhesion molecules	[64]
		Vitamin C	Oxidative DNA damage after DEP in vitamin C-supplemented guinea pigs	Oxidative DNA damage is formed independently of the vitamin C status after DEP	[66]
	Macrolides	EM, EM703	Effect of EM, EM703 on expression of IL-8-mediated NF-kB activation in HBEC	EM and EM703 inhibit expression of IL-8-mediated NF-kB activation caused by DEP	[74]
Endogenous antioxidants	Nrf2 activator	Sulforaphane	Effect of sulforaphane on IgE production in B cells	Sulforaphane can block DEP-induced enhanced IgE production in B cells	[78]
		Sulforaphane	Effect of sulforaphane on proinflammatory cytokines in HBEC	Sulforaphane can block DEP-induced proinflammatory cytokine production	[79]
		Sulforaphane	Effect of sulforaphane on nasal allergic response in humans	Sulforaphane attenuates nasal allergic response to DEP	[82]

Abbreviations: [*1]NAC, N-acetyl-L-cysteine; BUC, Bucillamine; Trx-1, Thioredoxin-1; OTC, Oxothiazolidine-4-carboxylic acid; EM, Erythromycin.
[*2]HBEC, human bronchial epithelial cells; OVA, ovalbumin; HUVEC, human umbilical vein endothelial cells.

Boosting the Endogenous Levels of Antioxidants

Sulforaphane is a naturally occurring isothiocyanate found in cruciferous vegetables and is most abundant in broccoli sprouts [75]. It is the most potent inducer of phase II enzymes identified to date and is thought to act via activation of the Nrf2 transcription factor [76, 77]. Overexpression of phase II enzymes by sulforaphane inhibits DEP-induced IgE production in B cells [78] and proinflammatory cytokine production in HBEC [79]; the use of sulforaphane has thus been proposed as a novel therapeutic approach [80]. Riedl et al. [81] reported on a placebo-controlled human study that demonstrated that oral sulforaphane can enhance phase II antioxidant enzyme expression in human airway. This study showed that the consumption of a broccoli sprout homogenate resulted in a dose-dependent increase in phase II enzyme RNA expression in nasal lavage cells, with maximum response at 200 grams. This is the first result to demonstrate clearly this biological effect of sulforaphane in vivo in the human airway. Furthermore, Heber et al. [82] recently reported a human study in which sulforaphane-rich broccoli sprout extract attenuated nasal allergic response to DEP.

Observations from the above studies highlight the importance of the Nrf2-antioxidant pathway and may provide chemopreventive strategies for many diseases and syndromes associated with oxidative stress from DEP exposure, not only for acute respiratory proinflammation. Because accelerated DNA adduct formation occurs in the lung of Nrf2 knockout mice exposed to DEP [30], it is possible that Nrf2 activators could decrease the susceptibility to DEP, as shown for accelerated DNA adduct formation in the lung, and therefore possibly counteract DEP-induced lung carcinogenesis.

Future Tasks

Chemoprevention by the above antioxidant drugs (Table 1) could be a future option for tailor-made prevention of DEP-induced adverse effects. On the basis of these findings, it is important to develop methods of identifying individuals susceptible to DEP-induced oxidative stress within large populations. It will be necessary to identify susceptible subpopulations in order to perform the targeted prevention of DEP-induced oxidative stress. The identification of *GSTM1* polymorphisms is a possibility, but it would be costly and present ethical concerns. Future studies should evaluate whether the measurement of airway inflammatory biomarkers in exhaled breath condensates (EBC) would be an appropriate method of assessment [83]. Alternatively, patients with diseases that Nrf2 helps to protect against [43-53] may be considered as a susceptible population to DEP-induced oxidative stress.

Increasing evidence supports the ability of antioxidants such as oral [84] or inhaled [85] NAC and oral sulforaphane [81] supplementation to moderate the effects of air pollution such as DEP among susceptible populations. More clinical research using human subjects is necessary to investigate antioxidant interventions as protective strategies for populations susceptible to air pollution such as DEP.

CONCLUSION

Antioxidants have been found to scavenge and detoxify free radicals and oxidants caused by DEP, to regulate glutathione biosynthesis and Nrf2 activation, and hence to inhibit antioxidant gene expression. It is therefore possible that the therapeutic administration or supplementation of multiple antioxidants and/or boosting of the endogenous levels of antioxidants will be beneficial as protective strategies for populations susceptible to air pollution such as DEP.

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