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Increased levels of etheno-DNA adducts and genotoxicity biomarkers of long-term exposure to pure diesel engine exhaust

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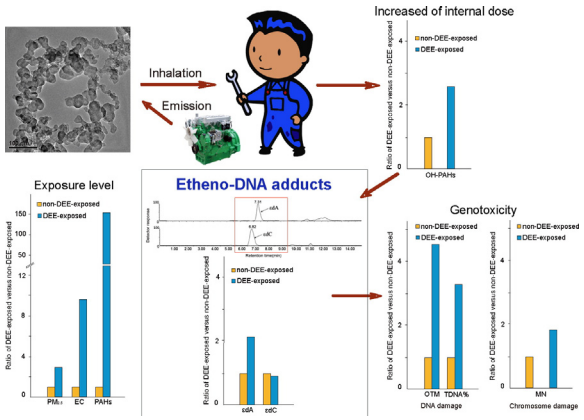
HIGHLIGHTS

- Higher urinary εdA level was found in the DEE-exposed workers.
- Urinary OH-PAHs were positively correlated with εdA.
- Increasing level of εdA was associated with increased genotoxicity biomarkers.

GRAPHICAL ABSTRACT



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ABSTRACT

Etheno-DNA adducts are biomarkers for assessing oxidative stress. In this study, the aim was to detect the level of etheno-DNA adducts and explore the relationship between the etheno-DNA adducts and genotoxicity biomarkers of the diesel engine exhaust (DEE)-exposed workers. We recruited 86 diesel engine testing workers with long-term exposure to DEE and 99 non-DEE-exposed workers. The urinary mono-hydroxylated polycyclic aromatic hydrocarbons (OH-PAHs) and etheno-DNA adducts (εdA and εdC) were detected by HPLC–MS/MS and UPLC–MS/MS, respectively. Genotoxicity biomarkers were also evaluated by comet assay and cytokinesis-block micronucleus assay. The results showed that urinary εdA was significantly higher in the DEE-exposed

Abbreviations: BER, base excision repair; BMI, body mass index; CBMN, cytokinesis-block micronucleus; DEE, diesel engine exhaust; DEP, diesel exhaust particles; EC, elemental carbon; GM, geometric means; GSD, geometric standard deviation; HDL, high-density lipoprotein; IARC, International Agency for Research on Cancer; LPO, lipid peroxidation; MN, micronucleus; NO, nitric oxide; OC, organic carbon; OTM, olive tail moment; OH-PAHs, mono-hydroxylated polycyclic aromatic hydrocarbons; PAHs, polycyclic aromatic hydrocarbons; PBLs, peripheral blood lymphocytes; $PM_{2.5}$, fine particulate matter; SCGE, single cell gel electrophoresis; TDNA%, percentage of tail DNA; 4-HNE, trans-4-hydroxy-2-nonenal; εdA, 1, N^6-ethenodeoxyadenosine; εdC, 3, N^4-ethenodeoxycytidine.

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workers ($p < 0.001$), exhibited 2.1-fold increase compared with the non-DEE-exposed workers. The levels of urinary OH-PAHs were positively correlated with the level of εdA among all the study subjects ($p < 0.001$). Moreover, we found that the increasing level of εdA was significantly associated with the increased olive tail moment, percentage of tail DNA, or frequency of micronucleus in the study subjects ($p < 0.01$). No significant association was observed between the εdC level and any measured genotoxicity biomarkers. In summary, εdA could serve as an indicator for DEE exposure in the human population.

1. Introduction

Diesel engine exhaust (DEE) is a complex mixture, consisting of gas phase and particle form. Diesel exhaust particles (DEP) consist of a core of elemental carbon (EC) and adsorbed organic compounds including polycyclic aromatic hydrocarbons (PAHs) (Wichmann, 2007). The aerodynamic diameter of most DEP ranges from 0.1 to 0.25 μm. This fine particulate matter ($PM_{2.5}$) is highly respirable and contributes to air pollution (Brunekreef and Holgate, 2002). Epidemiological studies have shown that the levels of the fine particulate air pollution in the environment including DEP are positively associated with mortality rates due to lung cancer and cardiopulmonary diseases (Dockery, 2001; Dockery et al., 1993; Gauderman et al., 2004; Pope, 2000; Pope et al., 2002). Consistently, lung cancer risk is increased among workers exposed to diesel exhaust (Guo et al., 2004; Sun et al., 2014), such as in the railroad (Garshick et al., 2004), in the trucking industry (Garshick et al., 2008), and in miners (Attfield et al., 2012; Silverman et al., 2012). Because of its association with lung cancer, DEE is classified as a Group 1 carcinogen (carcinogenic to humans) by the International Agency for Research on Cancer (IARC) (Benbrahim-Tallaa et al., 2012).

Many human cancers are related to an exposure of genotoxic chemicals, which cause DNA damage, resulting in the formation of DNA adducts and the alterations to DNA ultrastructure (Miller, 1970; Poirier, 2004). DNA bound carcinogen adducts indicates the amount of carcinogen which interacts with cellular macromolecules at the target site. DNA adducts formation has served as one of the process for human tumor induction, such as hepatocellular carcinoma and lung cancer (Ross et al., 1992; Tang et al., 2001).

Etheno-DNA adducts, such as 1, N^6-ethenodeoxyadenosine (εdA) and 3, N^4-ethenodeoxycytidine (εdC), are formed not only from exogenous carcinogens, such as vinyl chloride or urethane, but also endogenously by the reaction of DNA with products derived from lipid peroxidation (LPO) and oxidative stress (Chung et al., 1996; Swenberg et al., 1992). Urinary etheno-DNA adducts are an excretion of etheno DNA base adducts. εdA and εdC have been found to be present in human urine and explored as biomarkers for assessing oxidative stress (Chen et al., 2004; Hillestrom et al., 2004; Nair, 1999). Several studies have shown that the increased etheno-DNA adducts levels are correlated with certain diseases (Bartsch and Nair, 2000; Bartsch et al., 2011). Moreover, etheno-DNA adducts levels were significantly higher (6-fold) in spleen DNA of SJL mice injected with RcsX (pre-B-cell lymphoma) cells to over-produce nitric oxide (NO) (Gal et al., 1996; Nair et al., 1998). Although both εdA and εdC levels were significantly increased in the inflamed pancreatic tissue, only εdC was increased in the affected colonic mucosa of patients with Crohn's disease or ulcerative colitis (Nair et al., 2006). A recent study showed that 2-week exposure to DEE in mice led to an increased level of LPO (Yin et al., 2013). As previously referred, etheno-DNA adducts are formed by the reaction of DNA with active aldehydes, such as trans-4-hydroxy-2-nonenal (4-HNE), generated by LPO. However, the etheno-DNA adducts level in DEE-exposed workers is still unknown.

The comet assay and cytokinesis-block micronucleus (CBMN) assay are commonly used methods for monitoring the genotoxicity (Azqueta and Collins, 2013; Collins, 2009; Duan et al., 2009; Kassie et al., 2000). In comet assay, the olive tail moment (OTM) and the percentage of tail DNA (TDNA %) are used to measure DNA strand breaks. In mice, DEP exposure induced the increased levels of DNA strand breaks in the lung tissue (Kyjovska et al., 2015). Micronucleus (MN) is a biomarker to detect genomic instability, including chromosome breakage and chromosome loss, which serves as a strong predictor of lung cancer risk (Bonassi et al., 2007; Lloyd et al., 2013). A recent study found that the DEE-exposed workers exhibited significantly higher MN frequency compared with the non-DEE-exposed workers ($p < 0.001$) (Zhang et al., 2015). However, the relationship between etheno-DNA adducts and genotoxicity biomarkers is unknown and worthy of investigation.

Most of the studies related to DEE-induced early health effects have two significant hampers, including the limitation of exposure duration and the mixed exposure. In the healthy human volunteers exposure controlled studies, only the short term acute health effects of diesel exhaust exposure were observed. The occupational epidemiological studies in diesel exhaust exposed workers have been limited by the lack of quantitative exposure data and the mixed exposure of diesel exhaust with others, such as gasoline or outdoor air pollutants.

Although the DEE is carcinogenic to humans, a study of workers with long-term exposure to pure DEE is rare because of the lack of such a population. In the present study, we recruited diesel engine testing workers with a long-term exposure to DEE who performed their work in an indoor workplace with DEE as a major exposure. Meanwhile, we recruited non-DEE-exposed workers in a water factory as control population. We measured urinary mono-hydroxylated PAHs (OH-PAHs) to evaluate the DEE exposure level. Moreover, urinary εdA and εdC were detected to investigate the impact of DEE exposure on oxidative DNA damage. Furthermore, we analyzed the association between urinary OH-PAHs with etheno-DNA adducts. Finally, in order to illustrate the effect of DEE exposure on genotoxicity, the relationship between etheno-DNA adducts and OTM, TDNA %, or the frequency of MN in peripheral blood lymphocytes (PBLs) were also assessed. Our study revealed that εdA, but not εdC, was correlated with OH-PAHs and genotoxicity biomarkers. The disadvantages of the former studies conducted among workers exposed to DEE are the short exposure duration and the mixed exposure. Our results provide the first evidence of a link between etheno-DNA adducts and genotoxicity biomarkers after long-term exposure to pure DEE.

2. Materials and methods

2.1. Study subjects and sample collection

This study was approved by the Research Ethics Committee of the National Institute for Occupational Health and Poison Control, Chinese Center for Disease Control and Prevention (China CDC), and all participants signed an informed consent. The study population was previously described (Zhang et al., 2015). Briefly, the DEE-exposed workers performed their work to test heavy-duty diesel engines for at least one year in the DEE exposure workshop. The engine testing workshop was semi-enclosed and the major exposures were DEE and noise. The control group was water pump operator working in the same city. Exclusion criteria were participants with chronic diseases, acute infection, or those who had been exposed to X-ray in the preceding three months. All study subjects were interviewed by an occupational physician using a detailed questionnaire that included demographic information, smoking habits, alcohol consumption, work year, and personal medical