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Biodiesel versus diesel exposure: Enhanced pulmonary inflammation, oxidative stress, and differential morphological changes in the mouse lung

Naveena Yanamala [a], Meghan K. Hatfield [a], Mariana T. Farcas [a], Diane Schwegler-Berry [a], Jon A. Hummer [c], Michael R. Shurin [e], M. Eileen Birch [d], Dmitriy W. Gutkin [e], Elena Kisin [a], Valerian E. Kagan [f], Aleksandar D. Bugarski [c], Anna A. Shvedova [a,b,*]

[a] *Pathology & Physiology Research Branch/NIOSH/CDC, Morgantown, WV 26505, USA*
[b] *Department Physiology and Pharmacology, WVU, Morgantown, WV 26505, USA*
[c] *Office of Mine Safety and Health Research/NIOSH/CDC, Pittsburgh, PA 15236, USA*
[d] *NIOSH/CDC, 4676 Columbia Parkway, Cincinnati, OH 45226, USA*
[e] *Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA, USA*
[f] *Department of Environmental and Occupational Health, University of Pittsburgh, PA, USA*

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ABSTRACT

The use of biodiesel (BD) or its blends with petroleum diesel (D) is considered to be a viable approach to reduce occupational and environmental exposures to particulate matter (PM). Due to its lower particulate mass emissions compared to D, use of BD is thought to alleviate adverse health effects. Considering BD fuel is mainly composed of unsaturated fatty acids, we hypothesize that BD exhaust particles could induce pronounced adverse outcomes, due to their ability to readily oxidize. The main objective of this study was to compare the effects of particles generated by engine fueled with neat BD and neat petroleum-based D. Biomarkers of tissue damage and inflammation were significantly elevated in lungs of mice exposed to BD particulates. Additionally, BD particulates caused a significant accumulation of oxidatively modified proteins and an increase in 4-hydroxynonenal. The up-regulation of inflammatory cytokines/chemokines/growth factors was higher in lungs upon BD particulate exposure. Histological evaluation of lung sections indicated presence of lymphocytic infiltrate and impaired clearance with prolonged retention of BD particulate in pigment laden macrophages. Taken together, these results clearly indicate that BD exhaust particles could exert more toxic effects compared to D.

Introduction

Despite the widespread use of petroleum-based diesel (D) fuels, interest in vegetable oils as an alternative fuel source was reported in several countries as early as the 1920s and 1930s. The potential interest in alternative fuels was not evidenced until the fuel-energy crisis in the late 1970s and early 1980s, after which vegetable oil derived fuels gained their prominence as a potential alternative energy source (Hill et al., 2006; Ragauskas et al., 2006). One of the key issues of biodiesel (BD) use is to reduce the emissions of particulate matter (PM) and greenhouse gasses (GHG). The combustion of vegetable oil-derived biodiesel fuels was proven effective in producing similar or less emissions compared to petroleum-based D (Koonin, 2006). Regardless of its broad use in different operational areas, including transportation (on- and off-road vehicles), and other manufacturing/production (mining, oil and gas industry) sectors, inadequate attention has been paid to the possible health hazards of BD (Bunger et al., 2007; Krahl et al., 2001; Swanson et al., 2007).

Exposure to diesel exhaust in humans has been shown to cause a number of adverse health outcomes. For instance, acute exposure to diesel particulate matter (DPM) was shown to facilitate pulmonary inflammation with influx of phagocytic cells (Holgate et al., 2003a, 2003b), while long-term exposure was strongly associated with a greater incidence of cough, phlegm, and chronic bronchitis (Pronk et al., 2009). Additionally, exposure to DPM has been associated with

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* Corresponding author at: Pathology and Physiology Research Branch (MS-2015), 1095 Willowdale Road, Morgantown, WV 26505, USA. Fax: +1 304 285 5938.

E-mail addresses: wqu1@cdc.gov (N. Yanamala), wla4@cdc.gov (M.K. Hatfield), woe7@cdc.gov (M.T. Farcas), qzh3@cdc.gov (J.A. Hummer), shurinmr@upmc.edu (M.R. Shurin), mib2@cdc.gov (M.E. Birch), dwgutkin@hotmail.com (D.W. Gutkin), edk8@cdc.gov (E. Kisin), kagan@pitt.edu (V.E. Kagan), zjl1@cdc.gov (A.D. Bugarski), ats1@cdc.gov (A.A. Shvedova).

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a number of long-term adverse effects, such as exacerbation of pre-existing lung disease, respiratory infections, bronchoconstriction and cancer (Sawyer et al., 2010; Silverman et al., 2012). High-molecular weight polycyclic aromatic hydrocarbons (PAHs), and PAH-derivatives, such as nitro-PAHs from combustion exhaust are considered to be potent mutagens and carcinogens (Garshick et al., 2004; Tokiwa and Ohnishi, 1986). Additional adverse effects of DPM may also include vascular damage, cardiac malfunctions and pro-thrombotic effects (Mills et al., 2005, 2007; Nemmar et al., 2007; Rivero et al., 2005; Tornqvist et al., 2007). Clinical studies involving humans have shown impairment of vasodilation, and increased incidence of arrhythmias and myocardial infractions along with changes in heart rate (Hazari et al., 2011; Mills et al., 2005, 2007; Nemmar et al., 2009; Peretz et al., 2008; Watkinson et al., 1998).

BD derived from vegetable oils of various sources (rapeseed, soybean, corn, sunflower, etc.), is gaining popularity as an alternative fuel for its use in diesel engines to meet the requirements of the Clean Air Act. Currently the majority of BD fuels are produced by the trans-esterification of vegetable oils with methanol or another alcohol, generating fatty acid alkyl esters (Knothe et al., 2010). The major components of these oils are mono-unsaturated oleic acid (C18:1) and polyunsaturated linoleic acid (C18:2) and linolenic acid (C18:3) that are susceptible to oxidation upon combustion leading to formation of peroxides and a variety of secondary oxidation products (Knothe, 2005; Song et al., 2000). A detailed list of fatty acids composition of BD from various sources can be found in Supplemental Materials (Table S1). Combustion of BD was shown to reduce mass emissions of PM, unburned hydrocarbons (HC), PAHs, nitrogen oxides levels, carbon monoxide, and aldehyde like compounds (Garshick et al., 2004; Tokiwa and Ohnishi, 1986). Previous published studies showed the potential of neat BD and BD blends (Bugarski, 2006; Bugarski et al., 2010; McDonald et al., 1997) to lessen occupational exposures to elemental carbon (EC) and non-volatile fractions of DPM (Purcell et al., 1996). However, BD was found to increase particle-bound volatile organic fraction of PM and carbonyl emissions (Liu et al., 2009; Purcell et al., 1996). The semi-volatile fractions of BD exhaust demonstrated increased cellular toxicity compared to that of PM (Liu et al., 2009). Additional studies have compared the acute toxicity exerted by BD and D emissions using mutagenicity assays (Bunger et al., 1998). While the emissions of BD are less mutagenic than D with high sulfur content fuel (Kado and Kuzmicky, 2003), recent studies, including our own, demonstrated that BD is more mutagenic when compared to ultralow sulfur diesel fuel (ULSD) (Bunger et al., 2007; Kisin et al., 2013; Krahl et al., 2009). The non-methylated esters of BD were shown to be more mutagenic than both methylated BD and petroleum D (Bunger et al., 2000). Further, Ackland et al. has shown that exposure to 80% and 20% BD blends increased formation of multinucleated cells by 16% and 52%, respectively (Ackland et al., 2007). Studies by Brito et al. (2010) suggest that BD displays equal and/or more toxic effects compared with D fuel. While exposure to D, neat BD and a 50% BD blend induced lung inflammation in rats 1 h post exposure, no disparity between groups exposed to different fuels was found (Brito et al., 2010). These results were similar to those reported for the chronic BD exposure studies (Finch et al., 2002), where no difference between exposure of animals to the combustion of these different fuels was found.

As reported previously, differences in the toxicity induced by BD could be due to several factors including the chemical composition of BD, different additives used in engines, age and operating conditions of the engine (Obert, 1973; Ullman, 1989). These parameters could in turn facilitate a number of varied health outcomes found in occupational settings (Groves and Cain, 2000; Pronk et al., 2009). Considering BD fuel consists of unsaturated fatty acids, which are easily oxidized during combustion, we hypothesize that BD exhaust particles could induce pronounced adverse outcomes compared to D.

Materials and methods

Animals. Specific pathogen-free adult female C57BL/6 mice (8–10 weeks) were supplied by Jackson Laboratories (Bar Harbor, ME) and weighed 20.0 ± 1.9 g when used. Animals were housed one mouse per cage receiving filtered high efficiency particulate air (HEPA) in the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International-accredited National Institute of Occupational and Safety Health (NIOSH) animal facility. All animals were acclimated in the animal facility under controlled temperature and humidity for one week prior to use. Beta Chips (Northeastern Products Corp., Warrensburg, NY) were used for bedding which was changed weekly. Animals were supplied with water and certified chow 7913 (Harlan Teklad, Indianapolis, IN) ad libitum, in accordance with the guidelines and policy set forth by the Institute of Laboratory Animal Resources, National Research Council. All experimental procedures were conducted in accordance with a protocol approved by the NIOSH Institutional Animal Care and Use Committee (IACUC).

Details of exhaust/emission generation system and fuels. The DPM samples were collected at the diesel laboratory at NIOSH Office of Mine Safety and Health research (OMSHR). The exhaust samples were collected using a single batch of neat corn-based fatty acid methyl ester (FAME) BD, acquired from Peter Cremer (Cincinnati, OH, NEXSOL BD-100) and single batch of petroleum-based ultralow sulfur diesel (ULSD) fuel, acquired from a local supplier. The BD fuel met ASTM D 6751 standard. The samples of BD and D a particulates were collected from the exhaust of a mechanically controlled, naturally-aspirated directly injected Isuzu C240 (Isuzu Motors Limited) diesel engine equipped with diesel oxidation catalytic converter (DOC, Lubrizol, New Market, ON). The specifications for the engine are given in Table S2. The engine was coupled to a water-cooled eddy-current dynamometer from SAJ (Pune, India, Model SE150). The engine was exercised over four steady-state operating conditions (Table S3). These four loads are part of the International Standards Organization for Standardization (ISO) 8-mode test cycle ISO 8178 C1 (ISO, 1996). The exhaust particles originating from the four different loads were collected and combined for performing the toxicity studies as described in this study.

DPM sample collection system. A high volume sampling system was developed to advance the methods of collecting representative samples of diesel particulates for toxicity analysis. This system allows for collecting nano-sized and ultrafine DPM aerosols in liquid media, therefore preserving to the highest possible level physical and chemical characteristics of sampled aerosols. Collecting and assaying particulates in water minimizes non-physiologic agglomeration, dissolution, and surface conditioning of particulates> and destruction of the particulate properties that can occur in filter collection, solvent extraction, or the preparation of collected material. The diesel particulate samples were collected from partial-flow dilution system (dekati, Model FPS-4000) using custom designed sampling system made with a version of a versatile aerosol concentrator enrichment system (VACES) (Khlystov et al., 2005; Kim et al., 2000; Sioutas et al., 1999) (see Fig. S1) and BioSampler® (SKC, Eighty Four, PA) (see Fig. S1). The VACES system was used to grow diesel aerosols by condensing deionized water on surface of those aerosols. The VACES system was operated with one condenser column, one virtual impactor, and one BioSampler®. The diluted exhaust with temperatures ranging between 295 K and 305 K (function of the engine operating condition) was flown above body of deionized water heated at ~315 K. The water was condensed on the DPM in a single chilled condenser column. The temperature of the coolant in the chiller was maintained at 268 K. As a result of the condensation process, the median diameter of the newly generated aerosol was ~5 μm. The enlarged aerosols were separated from the rest of flow using a virtual impactor (VI). The major VI flow of 55 l/min was maintained using mass flow controller (Sierra Instruments, Model 850) and vacuum