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Effects of diesel exhaust particles on the condition of mouse ocular surface

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ABSTRACT

In order to evaluate the effects of diesel exhaust particles (DEP) on the ocular surface, different concentrations (100 and 1000 μg/ml) of DEP eye drops were administered on the mouse ocular surface for a period of 28 days. After DEP treatment, the corneal epithelial permeability to Oregon Green Dextran was studied, which increased proportionally with time. Also, the number of corneal epithelial cell layers significantly increased, which was accompanied with a high Ki67 expression. On the other hand, the number of goblet cells in the conjunctival fornix were reduced, and apoptotic cells were detected in the corneal and conjunctival epithelium by TUNEL assay in the DEP treated group, along with increased Caspase 3/8 expression. Furthermore, the number of CD4 positive cells significantly increased in the conjunctiva, while NF-κB p65 (phospho S536) expression was elevated in the cornea and also the conjunctiva. Our data revealed that the topical administration of DEP on the ocular surface in mouse disrupted the organized structure of the ocular surface and induced an inflammation of the cornea and conjunctiva.

1. Introduction

With the growing use of automobiles, the vehicle emissions are significantly contributing to the urban traffic air pollution. The impact of air pollution on human activities and human health has gained a worldwide concern. Particulate matters (PM), also called particle pollution are mixtures of solid particles and liquid droplets found in the air that are the main contributors to the air pollution. According to estimates released by the World Health Organization in 2007, airborne particles are responsible for half a million premature deaths each year (Robertson et al., 2012). Exhaust from diesel engines that is composed of particles of different sizes, is a large environmental health risk (Lucking et al., 2011). The on-board PM2.5 emission factors for heavy-duty diesel trucks with Euro III emission standard measured in China was on an average about 0.52 ± 0.55 g/kg fuel to 0.09 ± 0.10 g/km/vehicle (Huo et al., 2012). In 2017, 45% of ambient PM2.5 in Beijing was contributed by the vehicles, in which the diesel-powered trucks stood out as the worst polluters (www.bjepb.gov.cn). Diesel exhaust particles (DEP) can not only cause respiratory diseases like asthma, bronchitis, and rhinitis (Ghio et al., 2012) but also can affect the circulatory system, the reproductive system and the immune system (Pierdominici et al., 2014). It also contains mutagens and carcinogens that could penetrate the human respiratory system (Spycher et al., 2015).

The impact of DEP on the ocular surface has also been studied extensively, but not elucidated in detail. Since the eye is an organ that is directly in contact with the environment, the environmental quality is critical for the physiological function of the ocular surface (Dulull et al., 2016). DEP concentration is known to be directly proportional to the incidence of conjunctivitis in children (Van Roosbroeck et al., 2008). People residing in the urban regions exhibited more ocular surface symptoms than those in unpolluted regions. Increased air pollution led to itching, red eyes, epiphora, a shortened tear film break-up time and other ocular symptoms (Novaes et al., 2010). Frequent exposure to automobile exhaust gas is known to alter the inflammatory factors along with mucin and other ingredients in the tear, resulting in the decreased stability of the tear film (Torricelli et al., 2014). The mechanism by which DEP induces ocular surface damage remains elusive. The current study aimed to evaluate the ocular surface changes after DEP exposure and to explore its possible underlying mechanism. To the best of our knowledge, this is the first in vivo animal study to evaluate the effect of DEP on the ocular surface.

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2. Materials and methods

2.1. Diesel exhaust particle suspension

Standard diesel exhaust particulate (DEP; National Institute of Standards and Technology's Standard Reference Material SRM 2975) was used in the present study. To prepare a suspension of DEP, 1 mg of dry DEP was suspended in 1 ml 0.9% saline. The pre-mixed solution was subsequently sonicated for 20 min at 37 kHz at 37 °C. The stock suspension of DEP was prepared ex tempore, and diluted with normal saline to a working concentration of 0 , 100 , 1000 μg/ml (Tabor et al., 2016). The concentrations of PAH and Nitro-PAH in DEP are listed in Supplemental Table 1.

2.2. Characteristic of DEP

The surface morphology of DEP was observed by a field emission using Scanning Electron Microscope (SEM, JSM, Japan). All samples were sputter-coated with gold before SEM analysis. For analysis using the Inductively Coupled Plasma Mass Spectrometry (ICP-MS), 10 mg of DEP was weighed using analytical balance (SARTORIUS BT125D, accuracy 0.01 mg) and placed in a clean microwave digestion tank (Shanghai Yi Yao Technology Development Co., Ltd). 4 ml of 65% HNO_3 (Merck, AR) and 2 ml of 35% H_2O_2 (Xilong scientific, AR) was added and left for cold digestion for 1 h. The mixture was then set for the microwave digestion, (Power: 1200 W, Temperature: 120 ℃, Pressure: 45 atm, Holding time: 4 min). Temperature program protocol was referred from an earlier study (Cao et al., 2012). After cooling to room temperature, the digestion tank was opened and the digested solution was transferred to the syringe. The digestion tank was washed with MilliQ (MQ) water several times and the rinsing solution was transferred to the syringe with 0.22 μm filter, the filtrate was collected in 50 ml PP tube (syringe and PP tube was soaked in 5% HNO_3 for 24 h, and then cleaned with MQ water) and 20 ml volume was subjected to testing. In the same way, the empty cans were operated to obtain a blank control. 17 element were analyzed within a reasonable working linear range, including K, Ca, Mg, Fe (0–5000 ng/ml) ; Ag , Al , As , Ba , Be , Cd , Co , Cr , Cu , Mn , Mo , Ni , Pb , Sb , Se , Th , Tl , U , Zn (0–50 ng/ml). ICP-MS instrument main operating parameters are listed in Supplemental Table 2.

2.3. Experimental animals

Forty male C57BL/6 mice (18–20 g; procured from Nanjing Biomedical Research Institute of Nanjing University, China) were used for this study. All animals were allowed to acclimate to the local conditions for at least 1 week and were held in the standard environment throughout the study at room temperature 25 °C ± 1 °C, relative humidity 60% ± 10%, on a 12:12 light-dark hour cycle, with the availability of food and water. All procedures were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the protocols in this study were approved by the animal ethics committee of Xiamen University School of Medicine (approval ID: XMUMC2016-08-03).

2.4. Experimental procedures

According to previous in vitro studies (Tau et al., 2013), the mice were randomly divided into four groups: blank control group (n = 4), control group (n = 12), low-DEP group (n = 12), and high- DEP group (n = 12). The blank control group was untreated, the control group, low-DEP group, and high-DEP group were topically administered with saline, 100 and 1000 μg/ml DEP, respectively, with the dosage of 5 μL, twice daily (9 a.m., 9 p.m.) for 14 or 28 days. Corneal OGD (Oregon Green Dextran) staining was performed on day 0, 14, and 28. The ocular and orbit tissues were harvested carefully on day 14 and 28 for histologic examination, immunostaining, real-time reverse transcriptase-polymerase chain reaction (RT-qPCR), and western blot analysis.

2.5. Oregon green dextran staining

Corneal staining was performed before and after 14 days and 28 days treatment, using Oregon Green Dextran (OGD-488) - a conjugated fluorescent dye of a 70 kDa molecular size (Invitrogen-Molecular Probes, Eugene, OR, USA) as described previously (Gerondakis et al., 2014). In brief, 0.5 μL of OGD was instilled on the cornea, 1 min before euthanasia. Mice were executed by cervical dislocation. Eyes then were rinsed with 5 ml of 1 × PBS. Excess liquid was blotted carefully from the ocular surface with filter papers without touching the cornea. Digital pictures of both eyes were taken under 470 nm excitation and 488 nm emission wavelengths using a Nikon (Tokyo, Japan) AZ100 stereo- fluorescent microscope, with an exposure time of 2 s. Both eyes from each animal were evaluated; the right eye always first followed by the left eye. The mean intensity in the central cornea was evaluated by capturing digital images using NIS Elements (version 3.0) by placing a fixed region of interest (2-mm diameter circle) on the central cornea. The mean intensity of the fluorescence was read by the software.

2.6. Hematoxylin and eosin staining and periodic acid schiff assay

Hematoxylin and eosin (H&E) staining was performed in paraffin section. Three whole orbit tissue in each group were embedded in paraffin, cross-sectioned, and stained with PAS reagents (PAS Staining System 395B-1 KT; Sigma-Aldrich, St. Louis, MA, USA) and hematoxylin. The number of goblet cells in the conjunctival fornix was counted in six representative slices of homologous positions from each orbit tissue. These sections were examined using the light microscope (Eclipse 50i; Nikon Instruments, Tokyo, Japan). Goblet cell density was determined by counting PAS-positive cells as described before (Zhang et al., 2014).

2.7. TUNEL assay

Cellular apoptosis was detected using the TUNEL assay. The Dead End Fluorometric TUNEL System (G3250; Promega, Madison, WI) was employed in the study, and the cryosections of the cornea and conjunctiva were subjected for analysis as per the manufacturer's instructions. The tissue was then stained with DAPI (Vector Laboratories, Burlingame, CA). Photos were taken by Leica microscope (DM2500; Leica Microsystems, Wetzlar, Germany).

2.8. Immunostaining

Immunostaining was performed according to the previously published protocol (Li et al., 2016). On day 28, six of the whole globe tissues were embedded in optimal cutting temperature (OCT) compound (Tissue-Tek; Miles Inc., Elkhart, IN, USA) and frozen at − 80 °C. Frozen sections that were OCT-embedded (6-μm thick) and were sectioned using a cryotome (CM 1850UV; Leica Microsystems AG, Wetzlar, Germany) and stored at − 80 °C. The sections were fixed in acetone and permeated with 0.2% Triton X-100.

For immunofluorescent labeling, sections were blocked and were incubated at 4 °C overnight with rabbit polyclonal antibody of caspase 3 (1:300; ab49822, Abcam, Cambridge, UK), caspase 8(1:300; ab25901, Abcam) and Ki67 (1:200; ab15580, Abcam). After further incubation with AlexaFluor 488–conjugated goat anti-rabbit secondary antibody (1:1000; A-11034, Invitrogen, Carlsbad, CA, USA), sections were rinsed, counterstained with 4′,6-diamidino-2-phenylindole (DAPI), mounted, and photographed using a confocal laser scanning microscope (Fluo View 1000; Olympus Corp., Tokyo, Japan).

For immunohistochemical staining, as previously described (Li