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In vitro effects induced by diesel exhaust at an air–liquid interface in a human lung alveolar carcinoma cell line A549

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ABSTRACT

The present study examined the effects induced *in vitro* in human adenocarcinoma-derived alveolar basal epithelial A549 cells by diesel particulate matter (DPM) administered into the culture medium or by diesel exhaust administered at an air–liquid interface. When A549 cells were exposed to DPM in the culture medium, cell proliferation was inhibited at doses of 10–100 μg/mL; generation of interleukin (IL)-8 and the antioxidant enzyme, heme oxygenase-1 (HO-1), were inhibited at a dose of 100 μg/mL, and hydroxyl radicals were produced, but could be inhibited by catalase or superoxide dismutase. In contrast, when A549 cells were exposed to diesel exhaust, cell proliferation was inhibited in the absence, but not in the presence, of a diesel particulate filter (DPF); in the absence of a DPF IL-8 was produced in the same amount as in the control cells but was suppressed in the presence of a DPF; *HO-1* mRNA was transiently over-expressed in the presence of a DPF, and it was also increased slightly produced in the absence of a DPF but statistically not significant in the presence of a DPF, and it was also increased slightly produced in the absence of a DPF but statistically not significant; HO-1 was transiently produced independent of the absence or the presence of a DPF; and hydroxyl radicals were weakly produced, even in the presence of a DPF but could be inhibited by catalase or superoxide dismutase. It is thus suggested that oxidative stress may be induced by exposure to DPM or diesel exhaust and thereby exerts cytotoxic effect. The introduction of a DPF is effective to protect cells from the toxicity of diesel exhaust presumably by suppression of an oxidative stress.

1. Introduction

It has been considered that diesel exhaust may be harmful for the human respiratory system, for instance, by causing asthma (Ghio et al., 2012; Inoue and Takano, 2011). The International Agency for Research on Cancer (IARC) classified diesel exhaust as carcinogenic to humans (group 1) in June 2012 (Anonymous, 2012). The Tokyo Metropolitan Government regulates diesel vehicles under the Ordinance on Environmental Preservation to Secure the Health and Safety of Citizens of the Tokyo Metropolitan Area (commonly known as the Environmental Preservation Ordinance). Currently, diesel vehicle users in the Tokyo Metropolitan area must fit their vehicles with a diesel particulate filter (DPF) because, according to the ordinance, diesel vehicles cannot be driven unless the generation of particular matter (PM) is effectively reduced. The details of the regulation and its accomplishment can be found at the website of the Tokyo Metropolitan Government (http://www.kankyo.metro.tokyo.jp/en/automobile/diesel.html).

While a number of reports assessing the biological effects of diesel exhaust are in the literature, including our previous report indicating adverse effects on rat fetus (Watanabe and Kurita, 2001), it has been hard to elucidate the inhalation toxicity of diesel exhaust and its underlying mechanisms *in vivo* using animals, chiefly because of the limited availability of experimental systems. Thus, it is reasonable to use *in vitro* experiments; however, test substances are administered into the culture medium in typical *in vitro* assays, which cannot fully mimic human exposure to diesel exhaust. The Cultex® system is a unique cell culture exposure device that makes it possible to administer gaseous test substances

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to cells at an air–liquid interface (Steinritz et al., 2013; Rach et al., 2014).

The present study assessed the effects of diesel particulate matter (DPM) administered into the culture medium and diesel exhaust administered at an air–liquid interface using the Cultex® system on human adenocarcinoma-derived alveolar basal epithelial A549 cells. In addition, the influence of a DPF on the effects of diesel exhaust also was determined.

2. Materials and methods

2.1. Cells and chemicals

Human adenocarcinoma-derived alveolar basal epithelial A549 cells were obtained from the American Type Culture Collection (Manassas, VA, USA). RPMI1640 medium, fetal bovine serum (FBS), and 0.25% trypsin-ethylenediamine tetraacetic acid (EDTA) were purchased from GIBCO® of Life Technologies Corp. (Grand Island, NY, USA). DPM (NBS 1650b) was obtained from the National Institute of Standards and Technology (Gaithersburg, MD, USA). WST-1 solution was purchased from Dojindo Molecular Technologies, Inc. (Mashiki'machi, Kamimashiki'gun, Kumamoto, Japan). DuoSet® IC human CXCL8/interleukin (IL)-8, human tumor necrosis factor (TNF)-α/TNFSF1A and human total heme oxygenase (HO)-1/HMOX1 ELISA Development kits were obtained from R&D Systems, Inc. (Minneapolis, MN, USA). The RNA isolation reagent, High Capacity RNA-to-cDNA kit, TaqMan® Gene Expression Master Mix, and TaqMan® Gene Expression Assays were purchased from Applied Biosystems® of Life Technologies Corp. (Carlsbad, CA, USA). The protein assay reagent was obtained from Bio-Rad Laboratories, Inc. (Hercules, CA, USA).

2.2. Exposure of A549 cells to DPM administered into the culture medium (Experiment 1)

2.2.1. Cell culture

A549 cells were maintained in RPMI1640 medium with 10% FBS at 37 °C in a humidified incubator with 5% CO_2 and 95% air. The cells were harvested using trypsin-EDTA, counted, seeded onto a plate as specified below, and cultured for 48 h. Then, the medium was changed to the assay medium (RPMI1640 medium without FBS), and the cells were prepared for the assessments as specified below. Each experiment was conducted in octuplicate.

2.2.2. Cell proliferation assay

A549 cells were seeded onto a 96-well plate at a density of 1×10^4 cells/well. DPM was diluted with dimethyl sulfoxide (DMSO) and administered into wells at doses of 0.1–100 μg/mL, while a DMSO was administered to the control group. The final concentration of DMSO is 1% in the medium. After incubating for 24 or 72 h, the cells were treated with WST-1 assay solution for 3 h. Then, the absorbance of the medium was measured at a wavelength of 450 nm to estimate the cell proliferation activity as a percentage against the vehicle group values.

2.2.3. Measurements of IL-8 and TNF-α

A549 cells were seeded onto a 24-well plate at a density of 40×10^4 cells/well. DPM at doses of 1.0–100 μg/mL or vehicle was administered into the wells. After incubating for 24 or 72 h, concentrations of IL-8 and TNF-α were estimated using ELISA according to the manufacturer's protocol and were standardized against the protein values obtained for the HO-1 assessment described below.

2.2.4. Measurement of HO-1

A549 cells were seeded onto a 24-well plate at a density of 40×10^4 cells/well. DPM at doses of 1.0–100 μg/mL or vehicle was administered into the wells. After incubating for 4 or 24 h, the cells were harvested using trypsin-EDTA, washed twice with phosphate-buffered saline (PBS), and lysed with lysis buffer (1 mM EDTA, 0.5% Triton X-100, and protease inhibitor in PBS). Then, the concentration of HO-1 was estimated by ELISA according to the manufacturer's protocol and standardized against the total protein in the lysate that was quantified by Lowry's method (Lowry et al., 1951).

2.2.5. Detection of reactive oxygen species

A549 cells were seeded onto a 24-well plate at a density of 40×10^4 cells/well. DPM at 100 μg/mL or vehicle was administered into the wells. After incubating for 24 h, the assay medium was collected for electron spin resonance (ESR) assessment to detect reactive oxygen species. The spin trap agent used was 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO), and the conditions of ESR were as follows: magnetic field and sweep width, 335 ± 5 mT; output, 5 mW; abnormal-conditions, 9.412 GHz; sweep time, 2 min; response time, 0.1 s; and amplification ratio, 3.2×10^2.

2.3. Exposure of A549 cells to diesel exhaust administered at an air–liquid interface (Experiment 2)

2.3.1. Generation of diesel exhaust

Diesel exhaust in the absence of a diesel particulate filter (DPF) was generated by a 309-mL engine (Model NFAD-50-EX, Yanmar Co., Ltd., Osaka, Japan) that was run at a speed of 2400 rpm and was diluted with clean air to achieve PM and nitrogen dioxide (NO_2) concentrations of 1.6 mg/m^3 and 4.6 ppm, respectively.

On the other hand, diesel exhaust in the presence of a DPF was generated by allowing diesel exhaust from the engine to pass through a DPF (ODP-S06, O-DEN Co., Ltd., Toyo, Koto, Tokyo, Japan) to achieve PM and NO_2 concentrations of 0.47 mg/m^3 and 4.0 ppm, respectively.

2.3.2. Air–liquid interface exposure system

The Cultex® CG system (Cultex® Laboratories GmbH, Hannover, Germany) was used as an air–liquid interface exposure system. A schematic of the system configuration is illustrated in Fig. 1.

2.3.3. Cell culture

A549 cells were removed from the flask, seeded onto an insert, which had an area of 0.9 cm^2 and was filled with RPMI1640 medium with 10% FBS, at a density of 5×10^4 cells/insert, and cultured for 7 days. Then, the cells were exposed on the plate of the Cultex®

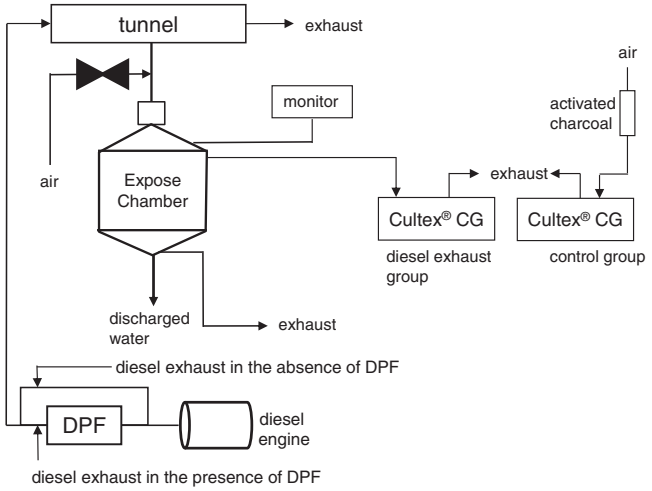

Fig. 1. Schematic of the configuration of the air–liquid interface exposure system.