Effect of diesel exhaust generated by a city bus engine on stress responses and innate immunity in primary bronchial epithelial cell cultures

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1. Introduction

Diesel exhaust (DE) exposures constitute a health risk for large populations living in urbanized areas (Laumbach and Kipen, 2012; Schmidt, 2016). Especially those suffering from chronic inflammatory lung diseases such as chronic obstructive pulmonary disease (COPD) and asthma may experience an increase in exacerbations during periods of high diesel exhaust exposure (Andersen et al., 2011; Weinmayr et al., 2013; Zarcone et al., 2016). However, a direct link between such DE-induced oxidative stress markers with disease exacerbations has not been established. In addition to air pollution, viral and bacterial infections are associated with COPD exacerbations (Vestbo et al., 2013). Non-typeable Haemophilus influenzae (NTHi) bacteria is commonly detected during acute COPD exacerbations, but is also present in patients with stable disease (Sethi and Murphy, 2008). Therefore, possibly DE exerts its effect on exacerbations through impairment of host defense against respiratory infections. The lung epithelium releases antimicrobial peptides and proteins such as human beta defensin (hBD)-2 and S100 calcium binding protein (S100A7) in response to bacterial infections (Hiemstra et al., 2015). Previously, impairment of such defenses by cigarette smoke was demonstrated and implicated in the increased susceptibility of smokers to respiratory infection (Herr et al., 2009; Pace et al., 2012). We have recently obtained evidence that DE may exert
similar effects of epithelial cell cultures, as shown by its ability to decrease induced hBD-2 expression (Zarcone et al., 2017). Another mechanism that may deregulate epithelial defense in COPD is provided by the observation of chronic activation of the integrated stress response (ISR) in COPD and other inflammatory lung diseases that may result from oxidants such as cigarette smoke as well as micro-organisms (Steiling et al., 2013; van t Wout et al., 2014). Therefore, detrimental effects of chronic activation of the ISR may also contribute to the effects of DE.

Air-liquid interface (ALI) cultures are a useful tool to investigate effects of exposure to different kind of toxic nanoparticles (Frieke Kuper et al., 2015; Kooter et al., 2013; Kooter et al., 2017). DE exposure of primary bronchial epithelial cells (PBEC), differentiated and exposed at the air-liquid interface (ALI) can better mimic the physiological composition of the lung epithelium in vitro. Effects of DE exposures on the antimicrobial response and the ISR of ALI-PBECs have been previously showed (Zarcone et al., 2016; Zarcone et al., 2017). Although these studies provided evidence for modulation of these mechanisms by DE, like most other studies they relied on the use of diesel exhaust emissions that can be compared to relatively high levels of ambient pollution. However, this may not fully represent the modern diesel engines that increasingly appear in traffic. Therefore, in the present study DE exposures were performed using emissions of city bus with Euro V diesel engine with selective catalytic reduction (SCR) catalyst using urea as reductant as after-treatment device, following the city-like Braunschweig testing cycle to mimic the pattern of acceleration typical for city traffic. We studied the cellular response of cells from (ex)smokers with a normal lung function and those with COPD to DE, and explored the modulation of bacteria-induced gene expression by DE to study modulation of innate immunity. Finally, we investigated the effect of DE generated by a cold engine (cold start) and compared this to that of a pre-heated engine.

2. Methods

2.1. Exposure to whole diesel exhaust generated by a Euro V bus engine

A typical Euro V bus engine (Cummins ISL 8.9 E5 280B) with maximal power of 209 kW was used at the TNO Automotive Powertrain Test Center (Helmond, the Netherlands) to produce diesel exhaust for all exposure sessions that were performed in a temporary biological research laboratory that was installed onsite. The 6 cylinders engine, with a cylinder displacement of 8.9 L, was connected to a selective catalytic reduction (SCR) device with urea dosing (after treatment device); no exhaust gas recirculation (EGR) system was present on the engine. During each exposure, the engine ran using commercially available (trade) fuel following an adapted Braunschweig city cycle (30 min per cycle), which is a typical bus city cycle (stop and go) that is commonly used for calibration purposes (Barlow et al., 2009). In addition, before the start of the experiment, the European transient cycle (ETC) test was performed to verify that the engine complies with the Euro V legislation. Experimental exposures were performed using a “warm” start set-up, following a first cycle of 30 min (“cold” start) during which the cells were not exposed. This design was followed to stabilize the emission and bypass a first burst of particulate matter (PM). In selected experiments, the effect of the cold start, in which particle concentrations were ~30% higher compared to normal operation (Fig. 1), was specifically addressed.

The diesel exhaust produced was first diluted in the constant volume sampler (CVS) tunnel with a constant volume flow of 3·10^4 L/min. The first dilution of DE (high, about 17 time diluted) was then collected in a central tank, to be directly addressed to the first Vitrocel® (Waldkirch, Germany) exposure module, or further diluted 2.5 or 6 times to generate the mid and low DE exposure conditions. Dilution of the DE was achieved by extracting DE from the central tank and adding mass flow controlled amounts of filtered humidified air. This exposure system allowed exposure of three inserts to the same condition at 37 °C with a flux velocity of about 5 mL/min. In the central tank, the exhaust temperature (T) and relative humidity (RH) were measured with a RH/T device (TESTO 635-1, TESTO GmbH & Co, Lenzkirch, Schwarzwald, Germany) to be 25.5 ± 1.1 °C and 58.6 ± 7.8%. The oxygen (O_2) level in the central tank was 19.9 ± 0.3% vol (mean value from two prolonged exposure sessions) as measured using an M&C PMA-10 oxygen analyzer (M&C Products Analysetechnik GmbH, Ratingen-Lintorf, Germany). On average, the carbon dioxide (CO_2) level in the central tank was 0.152 ± 0.02% as measured using a Vaisala GM70 probe with MI70 read-out unit (Vaisala, Helsinki, Finland).

Chemical characterization of the emissions has been performed as described previously (Jedynska et al., 2015). In brief in the first stage sampling points were used for: CO, CO_2, NOx and total hydrocarbons (THC) measurement using a gas analyzer (type MEXA-9100DREG, Horiba). Exhaust in the central tank, which is used for the cell exposure experiments, is also used for measurements of PM mass, polycyclic aromatic hydrocarbons (PAH) measurements using Teflon coated glass fiber filters in combination with the adsorbent amberlite XAD-2 resin (Rohm and Haas, Sigma Aldrich; PAH analysis only), as well as elemental carbon/organic carbon (EC/OC) analysis (Shetty et al., 2006).

2.2. Calculation of diesel dose

The particle size distribution in the central tank was measured using a TSI Scanning Mobility Particle Sizer (SMPS, model 3936L22 TSI Incorporated, Shoreview, MN, USA). The particle number size distribution was used to calculate the mass concentration of the aerosol using the effective density of diesel soot (Maricq and Ning, 2004). The calculated mass concentration in the central tank was 0.206 ± 0.015 mg/m^3 (n = 5). Delivered doses (DD), corresponding to the mass of particles applied to the inserts during the exposure time, was calculated as previously described (Zarcone et al., 2016), based on the SMPS measurements, the time of the exposure, the velocity flux and surface area of the insert, using the following formula:

\[ DD = \frac{I \times [PM] \times t}{S \times A} \]

In order to estimate the deposition efficiency of the particles in our system, five independent SMPS measurements of the first dilution of trade fuel emission were performed, to calculate the fractional efficiencies of particles based on the mass concentration (Maricq and Ning, 2004). The total deposition efficiency was thus estimated to be 1.9%. Deposited doses (dd), i.e. the actual mass of particles predicted to be deposited on the epithelial cell surface, were calculated based on the delivered doses and the deposition efficiency (dd = DD × 1.9%). PM concentrations, delivered doses and deposited doses for all exposure conditions are summarized in Table 1. The first dilution of the DE mixture produced during two independent sessions was used for the chemical characterization.

2.3. Cell culture

Primary bronchial epithelial cells (PBECs) were obtained from macroscopically normal lung tissue from patients undergoing resection surgery for lung cancer at the Leiden University Medical Center following the “Human Tissue and Medical Research: Code of Conduct for responsible use (2011)” guideline as previously described (Zarcone et al., 2016). Cells were used from 5 non-COPD and 7 COPD donors that were matched for age. COPD status was determined based on the patient’s lung function following the Chronic Obstructive Lung Disease (GOLD) classification (Vestbo et al., 2013): one GOLD III, five GOLD II and one GOLD I. Lung function data are shown in Table 2.

Following expansion, well-differentiated cultures were obtained by seeding passage 2 PBECs in 12 well-plate Transwell inserts (Corning Costar Corporation, Cambridge, MA) and feeding these every two days
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