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Transcriptional response to organic compounds from diverse gasoline and biogasoline fuel emissions in human lung cells

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ABSTRACT

Modern vehicles equipped with Gasoline Direct Injection (GDI) engine have emerged as an important source of particulate emissions potentially harmful to human health. We collected and characterized gasoline exhaust particles (GEPs) produced by neat gasoline fuel (E0) and its blends with 15% ethanol (E15), 25% n-butanol (n-But25) and 25% isobutanol (i-But25). To study the toxic effects of organic compounds extracted from GEPs, we analyzed gene expression profiles in human lung BEAS-2B cells. Despite the lowest GEP mass, n-But25 extract contained the highest concentration of polycyclic aromatic hydrocarbons (PAHs), while i-But25 extract the lowest. Gene expression analysis identified activation of the DNA damage response and other subsequent events (cell cycle arrest, modulation of extracellular matrix, cell adhesion, inhibition of cholesterol biosynthesis) following 4 h exposure to all GEP extracts. The i-But25 extract induced the most distinctive gene expression pattern particularly after 24 h exposure. Whereas E0, E15 and n-But25 extract treatments resulted in persistent stress signaling including DNA damage response, MAPK signaling, oxidative stress, metabolism of PAHs or pro-inflammatory response, i-But25 induced changes related to the metabolism of the cellular nutrients required for cell recovery. Our results indicate that i-But25 extract possessed the weakest genotoxic potency possibly due to the low PAH content.

1. Introduction

Air pollution has emerged as a worldwide problem and numerous studies have raised concerns about environmental and health effects of particulate matter (PM) in the atmosphere. A crucial portion of particles is derived from anthropogenic activities, such as the burning of fossil fuels in vehicle engines, domestic heating, power plants and industrial processes.

It has been demonstrated that long- and short-term exposure to the PM of aerodynamic diameter (d_{ae}) < 2.5 μm (PM2.5) can cause premature death and health disorders, such as adverse implications on the cardiovascular system and respiratory effects including asthma attacks (Pope III and Dockery, 2006). PM from anthropogenic activities consists of the aggregated nuclei composed largely of elemental carbon, with high concentrations of toxic substances adsorbed on the surface, such as acid sulphates, soluble metals, and organic compounds including carcinogenic polycyclic aromatic hydrocarbons (c-PAHs). The serious health risks are posed not only by PM2.5 but particularly by the ultrafine fraction (d_{ae} < 100 nm). PM2.5 are potentially more harmful than larger particles because they can deposit deeper into the lungs, and ultrafine particles may even penetrate into the bloodstream and reach target organs (Xia et al., 2016).

Motor vehicle emissions are among the major sources of air pollutants in many urban areas. Although emissions have been reduced over the last few decades due to the development of new fuels, improvement of engine exhaust after-treatment technology and due to the

Abbreviations: E0, Neat gasoline fuel; E15, Blend of gasoline and 15% ethanol; GDI, Gasoline Direct Injection; GEPs, Gasoline Exhaust Particles; i-but25, Blend of gasoline and 25% isobutanol; n-but25, Blend of gasoline and 25% n-butanol; PAHs, Polycyclic Aromatic Hydrocarbons

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increasingly stringent emission controls, the number of vehicles is still growing and studies of the toxic effects associated with exposure to vehicle engine exhaust are often complicated by a various composition of emissions. Modern gasoline engines with the innovative direct fuel injection system known as Gasoline Direct Injection (GDI) or Direct Injection Spark Ignition (DISI) offers numerous benefits including improved fuel efficiency and thus reduced CO_2 emissions, compared to conventional port fuel injection technology (PFI). Currently, emission limits for new cars are becoming more stringent every 3–5 years, so GDI is gradually replacing the historically dominant PFI engines. However, GDI engines also have several shortcomings such as the production of a substantial amount of particulate emission due to the direct fuel injection technology (Tripathy et al., 2017).

Increasing demands for alternative fuels produced from renewable sources attempting to reduce greenhouse gas emissions, energy dependency and to diversify energy resources have raised an interest in numerous biofuels and their blends. Apart from ethanol, the most prominent bioadditive worldwide, butanol might represent a competitive alternative offering further benefits (Jin et al., 2011). However, the use of bio-additives to supplement neat gasoline fuel may cause profound changes in emission properties such as particle morphology, size distribution and chemical composition, thus complicating the assessment of health and environmental risks. Importantly, compared to extensive research dealing with the toxicity of diesel and biodiesel fuel emissions, studies on the comparative toxicity of fossil gasoline and biogasoline exhaust particles or their organic extracts are very scarce. Acute exposure to emissions from neat gasoline and gasoline-ethanol blends was examined in human lung cells (Bisig et al., 2016). The authors found no adverse cell responses in this exposure; however, they pointed out that chronic and in vivo studies are missing. Moreover, no study exists on the toxicity of butanol-gasoline blends.

We recently reported a study on the comparative toxicity of organic compound mixtures extracted from diverse diesel and biodiesel particulate emissions in human lung BEAS-2B cells. Our results suggested that biofuels (conventional biodiesel fuel B100 and new generation of biodiesel fuel NexBTL) and the blend B30 considerably changed the particulate emission properties including chemical composition and affected various cellular processes on transcriptional level thus causing distinctive molecular responses in BEAS-2B cells (Libalova et al., 2016).

The present study addresses the important issue concerning the toxicity of particles emitted by gasoline direct injection engines. We aimed to compare chemical properties and toxic effects of organic extracts from GEPs produced by the combustion of conventional gasoline fuel and currently used (ethanol) or candidate (butanol) biofuels. Whole-genome gene expression profiling in human lung BEAS-2B cells was used to identify the major processes and genes which were commonly modulated after the exposure to all GEP extracts, as well as those specific for each treatment. This integrated approach, linking the characteristics of gasoline and alcohol-gasoline blends of particulate emissions with their toxic effects may improve the current knowledge of their impact on health and the environment.

2. Materials and methods

2.1. Chemicals and biochemicals

Agilent RNA 6000 Nano Kit was purchased from Agilent Technologies (Waldbronn, Germany), Bronchial Epithelial Basal Medium and BEGM™ BulleKit™ from Lonza (Basel, Switzerland); human bronchial epithelial cells BEAS-2B from ATCC (Manassas, VA, USA); dimethylsulphoxide (DMSO) from Sigma-Aldrich (St. Louis, MO, USA); WST-1 Proliferation Assay, Cytotoxicity Detection Kit (LDH) and High Fidelity cDNA synthesis Kit from Roche (Mannheim, Germany); NucleoSpin RNA II Isolation Kit from Macherey-Nagel (Düren, Germany); Illumina Human-HT12 v4 Expression BeadChips were from Illumina (San Diego, CA, USA); Illumina TotalPrep RNA Amplification Kit from Thermo Fisher Scientific (Waltham, MA, USA); Custom Designed Real-Time PCR Assay, qRT-PCR master mix and geNorm Reference Gene Selection Kit from Primerdesign (Southampton, UK); Standard Reference Material SRM 1650b (diesel particulate matter) from NIST (Gaithersburg, MD, USA).

2.2. Test vehicle, fuels and exhaust particles collection

The tests were carried on a typical European small family car (C-segment production passenger car), 2013 Ford Focus station wagon, with a three-cylinder 1.0 L turbocharged gasoline direct injection EcoBoost engine (92 kW @ 6000 rpm, 170 Nm @ 1400–4500 rpm, certified to Euro 6) and a 6-speed manual transmission, mileage of 7962 km (4948 mi) at the beginning and 10,130 km (6296 mi) at the end of the study.

Non-oxygenated gasoline with a nominal research octane number of 95, meeting ČSN EN228 specifications, was obtained at the local fuel station (EuroOil, Buštěhrad, Hřebečská 695, 27343), and used as the baseline fuel for the testing. Commercially available E85 fuel, also obtained from a local fuel station (LPG-AUTO s.r.o., Michelská 4/11, Prague 14000) and analyzed to contain 70% of ethanol, was mixed with the base fuel to produce a blend containing 15% of ethanol by volume (E15). Technical grade n-butanol (Chemlogistic, Pardubice) and isobutanol (Chemap, Dašice) were also mixed with the baseline fuel to obtain a blend of 25% of n-butanol with gasoline (n-But25) and a blend of 25% isobutanol with gasoline (i-But25). The fuels were metered on a mass basis using their actual densities into 20-liter canisters and splash-blended.

The vehicle was operated on a 4-wheel chassis dynamometer, according to the Common Artemis Driving Cycle, created to represent automobile driving patterns in Europe (André, 2004). The Artemis driving cycle comprises of an urban, rural and motorway section. Several versions of the motorway section, differing in maximum speed, are defined; the version with a maximum speed of 130 km/h (79 mph) was run here. The whole Artemis cycle was repeated four times to collect representative samples of exhaust particulate emissions.

The exhaust was routed into a full-flow dilution tunnel with a constant volume sampler (CVS) operating at 10.8 m^3/min (381 cfm), from which samples were taken for online measurements and offline analyses. The instantaneous dilution ratio, expressed as the ratio of the CVS flow and the instantaneous exhaust flow, varied considerably over the cycle and ranged from about 5:1 at full load to nearly 100:1 at idle. The online measurements included concentrations of hydrocarbons, CO, CO_2, NO_x and particle number concentrations. Particle size distributions were measured online with a fast mobility particle sizer (EEPS, Model 3090, TSI), preceded by a secondary dilution by a rotating disc diluter (MD-19, Matter Engineering) set to 180:1 dilution ratio; the diluter head was heated to 150 °C (Vojtisek-Lom et al., 2015a, 2015b).

For toxicity assays, diluted exhaust from the tunnel was sampled on 8″ × 10″ (203 × 254 mm) Teflon-coated glass fiber filters (Pall TX40HI20-WW), at a 67.8 m^3/h sampling rate using a pair of modified EcoTech 3000 high-volume samplers. The sample from the high-volume sampler was returned to the CVS; the sum of the remaining flows not returned to the CVS was added to the CVS nominal flow, and the emissions calculations were done with the corrected flow.

2.3. Particle characterization and chemical analysis

Organic compounds were extracted with dichloromethane in automated extraction apparatus Behr EF (BEHR, Germany) for 4 h. Aliquot parts of the crude extract were re-dissolved in the required volume of acetonitrile for HPLC/DAD and LC/MS-MS; and in dimethyl sulfoxide (DMSO) for bioassays. The method of external standardization was used for quantification of all PAH contaminants. The accuracy and precision of the analytical methods was determined by analysis of the standard