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Hazard identification of exhausts from gasoline-ethanol fuel blends using a multi-cellular human lung model

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ABSTRACT

Ethanol can be produced from biomass and as such is renewable, unlike petroleum-based fuel. Almost all gasoline cars can drive with fuel containing 10% ethanol (E10), flex-fuel cars can even use 85% ethanol (E85). Brazil and the USA already include 10–27% ethanol in their standard fuel by law. Most health effect studies on car emissions are however performed with diesel exhausts, and only few data exists for other fuels. In this work we investigated possible toxic effects of exhaust aerosols from ethanol-gasoline blends using a multi-cellular model of the human lung.

A flex-fuel passenger car was driven on a chassis dynamometer and fueled with E10, E85, or pure gasoline (E0). Exhausts obtained from a steady state cycle were directly applied for 6 h at a dilution of 1:10 onto a multi-cellular human lung model mimicking the bronchial compartment composed of human bronchial cells (16HBE14o-), supplemented with human monocyte-derived dendritic cells and monocyte-derived macrophages, cultured at the air-liquid interface. Biological endpoints were assessed after 6 h post incubation and included cytotoxicity, pro-inflammation, oxidative stress, and DNA damage. Filtered air was applied to control cells in parallel to the different exhausts; for comparison an exposure to diesel exhaust was also included in the study.

No differences were measured for the volatile compounds, i.e. CO, NO_x, and T.HC for the different ethanol supplemented exhausts. Average particle number were 6×10^2 #/cm^3 (E0), 1×10^5 #/cm^3 (E10), 3×10^3 #/cm^3 (E85), and 2.8×10^6 #/cm^3 (diesel).

In ethanol-gasoline exposure conditions no cytotoxicity and no morphological changes were observed in the lung cell cultures, in addition no oxidative stress - as analyzed with the glutathione assay - was measured. Gene expression analysis also shows no induction in any of the tested genes, including mRNA levels of genes related to oxidative stress and pro-inflammation, as well as indoleamine 2,3-dioxygenase 1 (*IDO-1*), transcription factor NFE2-related factor 2 (*NFE2L2*), and NAD(P)H dehydrogenase [quinone] 1 (*NQO1*). Finally, no DNA damage was observed with the OxyDNA assay. On the other hand, cell death, oxidative stress, as well as an increase in pro-inflammatory cytokines was observed for cells exposed to diesel exhaust, confirming the results of other studies and the applicability of our exposure system.

In conclusion, the tested exhausts from a flex-fuel gasoline vehicle using different ethanol-gasoline blends did not induce adverse cell responses in this acute exposure. So far ethanol-gasoline blends can promptly be used, though further studies, e.g. chronic and in vivo studies, are needed.

1. Introduction

Petroleum-based fuels will not last forever; renewable sources of energy are therefore needed. Already in the phase of combustion

Abbreviations: ALI, Air liquid interface; CO, Carbon monoxide; CVS, Constant volume sampling; E0, pure gasoline; E10, 10% ethanol, 90% gasoline; E85, 85% ethanol, 15% gasoline; GSH, Glutathione; LDH, Lactate dehydrogenase; LSM, Laser scanning microscope; MDDC, Monocyte-derived dendritic cells; MDM, Monocyte-derived macrophages; NO_x, Nitric oxides; PN, Particle number; SSC, Steady state (driving) cycle; T.HC, Total hydrocarbon; WLTC, Worldwide harmonized light vehicle test cycle

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motors invention and development, ethanol was in discussion as a fuel option. But it was not until the 1970s, when ethanol as a fuel supplement started to raise attention, first in Brazil, later also in the USA and Canada. Nowadays, more and more countries recognize ethanol as an interesting alternative or additive to petroleum-based fuels (Agarwal, 2007; Guarieiro and Guarieiro, 2013). Apart from pure gasoline (E0), different ethanol-gasoline blends are used, most prominently 10% ethanol and 90% gasoline (E10), which is the standard gasoline in the USA and planned for the EU by 2020 (EU, 2009), but also 85% ethanol and 15% gasoline (E85) is available, e.g. in Switzerland. While all newer cars can run on E10, special flex-fuel vehicles are needed for E85.

Ethanol acts as a fuel oxygenate, resulting in less particle mass (Chan et al., 2014) and particle number (PN) (Pirjola et al., 2015) in comparison to diesel or gasoline driven vehicles (Guarieiro and Guarieiro, 2013). However, ethanol can also be oxidized to acetaldehyde and further to acetic acid (Lopez-Aparicio and Hak, 2013), and also other aldehydes like formaldehyde and acrolein are expected to be increased in E85 exhaust (Massad et al., 1993). Such emissions are known to be harmful (e.g. Cancer (2006)) and possible effects need therefore to be carefully evaluated (Jacobson, 2007). Aldehydes for example have a very short half-life and are quickly transformed to reactive radicals in the presence of sunlight (Massad et al., 1993). Although research activities have increased during the last years to correlate emissions with engine type and fuel there is still only little data available on the risk assessment in terms of toxicity of ethanol supplemented fuel.

The human lung is especially vulnerable to air pollutants (Loomis et al., 2013) as shown by many epidemiological studies (Brunekreef and Holgate, 2002; Villeneuve et al., 2011; Simkhovich et al., 2008), as well as by human (Ghio et al., 2012; Klepczyńska-Nyström et al., 2012; Larsson et al., 2007; Nyström et al., 2010; Muala et al., 2015), animal (Mauderly et al., 2014; Pardo et al., 2015), and cell culture experiments (Hiraiwa and van Eeden, 2013; Kooter et al., 2013; Oeder et al., 2015; Schwarze et al., 2013; Cheng et al., 2003). Gasoline exhaust was not as extensively studied as diesel exhaust (Reed et al., 2008). The National Environmental Respiratory Center (NERC) published a series of papers on gasoline exhaust (Mauderly et al., 2014; Reed et al., 2008; McDonald et al., 2007). A subchronic gasoline exhaust exposure to rats and mice for 6 months revealed no general health effects like mortality, illness, or injury. However, increased DNA damage in the lung as well as increased cytotoxicity, measured by lactate dehydrogenase (LDH) in the bronchoalveolar lavage has been described. Interestingly a lot of the effects could be attributed to the gaseous phase of the exhaust (Reed et al., 2008). With regard to ethanol exhaust emissions, only studies in the late 80s were found which investigated the health effects of such exhausts (Bernson, 1983; Massad et al., 1985, 1986; Lotfi et al., 1990). Böhm and colleagues exposed rats to gasoline and ethanol exhaust for 5 week and found decreased expiratory flow after gasoline, but not after ethanol exhaust exposure and the most intense pathological lesions in the lungs were observed after gasoline exhaust exposure. Additionally, increased mutagenicity was measured with the micronucleus assay in mice exposed for 2 weeks to gasoline exhaust. These findings, among others, pointed to a chronic toxicity of gasoline exhaust, but not ethanol exhaust (Massad et al., 1986). In addition, the same group also showed that acute toxicity is significantly higher in gasoline than in ethanol exposed animals (Massad et al., 1985).

The attribution of a given subset of emissions to a certain adverse effect is complex but required when different fuels and/or engines are tested. Fast, low-cost, and reliable test systems are therefore needed to assess benefits and risks of these new technologies. For this purpose, the collection of condensable carbonaceous compounds and particle-extractable compounds is possible and is a potential research approach, which has been performed in previous studies (e.g. Che et al., 2010). But, in addition to be labor-intensive, this method does not consider non-condensable gaseous compounds (e.g. nitrogen oxides (NO_x)) and the extraction procedures are likely to affect the samples. A more accurate method consists of collecting the complete exhaust, including the particulate, condensed, and gaseous fraction. To address these issues we have developed a method which allows exposing human lung epithelial cells cultured at the air-liquid interface (ALI) directly to the complete engine exhaust. This system has already been used in the past for risk assessment of scooter (Muller et al., 2010), diesel (Steiner et al., 2012, 2013a. 2013b, 2014) and gasoline (Bisig et al., 2015) exhaust.

The aim of this study was to investigate the exhaust components produced from a passenger car with ethanol supplemented fuels ranging from E0, E10, and E85 and to correlate the emissions with possible effects in a multi-cellular human lung model. The lung cells were exposed to the exhaust at the ALI for 6 h and after a 6 h post-incubation biological endpoints such as cytotoxicity, pro-inflammation, oxidative stress, and mutagenicity were assessed. The effects were compared to filtered air as well as to cells exposed to diesel vehicle exhaust without a particle filter.

2. Materials and methods

2.1. Materials

All materials were purchased from Sigma-Aldrich unless otherwise stated.

2.2. Cell cultures

The multi-cellular human lung model composed of three cell types mimicking the bronchial compartment was used as previously described (Steiner et al., 2013a). Briefly, 16HBE14o- human bronchial epithelial cells (Cozens et al., 1994; Forbes et al., 2003) were seeded (2.4×10^5 cells/cm^2) on fibronectin-coated 6-well inserts (3 μm pores, BD Falcon) and cultured for 5 days. Human whole blood monocytes were isolated from buffy-coats provided by the blood donation service SRK Bern and purified using CD14 Microbeads (Milteny Biotech, Bergisch Gladbach, Germany) as described by Steiner et al. (2013b). Monocytes were differentiated to macrophages (MDM) and dendritic cells (MDDC) for 5–6 days and then added on top (1.2×10^4 MDM cells/cm^2) and on the bottom (2.0×10^5 MDDC cells/cm^2) of the insert, respectively. Differentiation agents were GM-CSF (Granulocyte macrophage colony-stimulating factor, Milteny Biotech) and IL-4 (Biotechne, R & D systems, Abington, United Kingdom) to obtain MDDC and M-CSF (Macrophage Colony-Stimulating Factor, Milteny Biotech) for MDM, all at a concentration of 10 ng/ml. One day after composition, the multi-cellular model was transferred to the ALI for another day before exposure to either filtered air or exhaust.

2.3. Test vehicle and exposure system

A modern gasoline flex-fuel passenger car (2012, Euro5a, gasoline direct injection, mileage during exposures was 10′000–15′000 km) was driven on a chassis dynamometer for 6 h. With the exception of the fuel, no changes to the car were made; inclusively standard lubricant oil (Castrol Magnatec 5 W-30) and the original three-way catalyst were used in all conditions. E85-fuel was purchased at a gas station and E10 was obtained by splash blending commercial E0 (RON 95) and E85. The fuels were stored at room temperature in 60 l barrels for less than 2 months. Unlike the mixture of diesel and non-esterified plant oils, ethanol and gasoline mix very well and no separation is expected.

A steady state cycle (SSC), consisting of five states (each 20 min with 95 km/h, 61 km/h, 45 km/h, 26 km/h, and idling) was driven; these velocities are derived from the Worldwide harmonized light vehicle test cycle (WLTC) (UNECE, 2016), representing the mean velocity of each of the four parts. In a 6 h exposure, the SSC was repeated 3 times, the fourth cycle was started until 6 h were completed.