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Basophil mediated pro-allergic inflammation in vehicle-emitted particles exposure

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ABSTRACT

Despite of the fact that engine manufacturers develop a new technology to reduce exhaust emissions, insufficient attention given to particulate emissions. However, diesel exhaust particles are a major source of air-borne pollution, contain vast amount of polycyclic aromatic hydrocarbons (PAHs) and may have deleterious effects on the immune system, resulting in the induction and enhancement of pro-allergic processes. In the current study, vehicle emitted particles (VEP) from 2 different types of cars (diesel - D and gasoline - G) and locomotive (L) were collected. Overall, 129 four-week-old, male SPF-class Kunming mice were subcutaneously instilled with either low dose 100, 250 or high dose, 500 mg/kg VEP and 15 mice were assigned as control group. The systemic toxicity was evaluated and alterations in the percentages of the CD3, CD4, CD8, CD16, CD25 expressing cells, basophils, eosinophils and neutrophils were determined. Basophil percentages were inversely associated with the PAH content of the VEPs, however basophil sensitization was more important than cell count in VEP exposure. Thus, the effects of VEP-PAHs emerge with the activation of basophils in an allergen independent fashion. Despite the increased percentage of CD4+ T cells, a sharp decrease in basophil counts at 500 mg/kg of VEP indicates a decreased inhibitory effect of CD16+ monocytes on the proliferation of CD4+ T cell and suppressed polarization into a Th2 phenotype. Therefore, although the restrictions for vehicles emissions differ between countries, follow up studies and strict regulations are needed.

1. Introduction

Vehicle emitted particulate (VEP) matter can have direct consequences for human and environmental health (Bonazza et al., 2007; Pope and Dockery, 2006). Although the vehicle gas emission and water soluble counterparts have been widely investigated, the effects of VEPs are ignored (Canagaratna et al., 2010; Cheung et al., 2009; Karjalainen et al., 2014). Exhaust gas is emitted as a result of the combustion of various kinds of fuels (Omidvarborna et al., 2014) and according to the type of the engine, it is discharged into the atmosphere and contributes to the air pollution (Golokhvast et al., 2015a, 2015b). Thus, the characteristics of the particulate matter that is emitted by each car differs. Caiazzo et al. indicated that 53,000 early deaths occur per year in the United States alone because of vehicle emissions (Caiazzo et al., 2013). Although the pollutants emitted by vehicles are typically regulated by governmental agencies, due to their undesirable effects, the interest in particulate matter (PM) emissions has grown substantially and subsequent issues arise regarding the necessity of regulations, in the last few years (Mazzoleni et al., 2010). In 2012, based on sufficient evidence, the International Agency for Research on Cancer

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(IARC) concluded that diesel engine exhaust exposure is associated with an increased risk for lung cancer. It is classified as Group 1, carcinogenic to humans (Attfield et al., 2012; Benbrahim-Tallaa et al., 2012; Silverman et al., 2012). Globally, the particle emission limitations for vehicles are under strong control. VEPs are restricted by standards which have quite wide variations between different countries. Thus, European Union restricted the VEPs total mass in 2009, and the number in 2014 (EC Treaty/Euratom Treaty, 2008; Martini et al., 2009). Although the bulk amount of investigations today is concentrated on the size of VEP and their chemical composition (Gillies, 2014; Hochmuth et al., 2013; Kalberer et al., 2014; Wichmann and Shapiro, 2006), it has been shown that the main particulate fraction of diesel exhaust can easily penetrate into the lung when inhaled, because of their small size as they mostly consist of fine particles. Also, their rough surfaces make it easy to bind to other toxins in the environment, thus increase their hazardous effects (Fenga et al., 2016; Omidvarborna et al., 2015; Piperigkou et al., 2016). On the one hand, it has been shown that diesel exhaust particles (DEP) are associated with the induction of free radical production and initiation of pro-inflammatory responses (Bai et al., 2001; Becker et al., 2005; Bostan et al., 2016; Salvi et al., 1999; Vitkina et al., 2016). On the other hand, DEP have been implicated in the increased incidence and morbidity of asthma and allergic rhinitis (Diaz-Sanchez et al., 1994; Lubitz et al., 2010). However, data regarding the gasoline and locomotive emitted particles are insufficient. Previously, our group determined the morphometric parameters and chemical composition of the VEPs from different car brands. This investigation revealed that the various size fractions and aerodynamic diameters may have differential spreading to the environment resulting in health consequences on exposed individuals (Golokhvast et al., 2015b; Sayapina et al., 2016). However, the exposure amounts differ between the countries or even in rural or urban areas (Gramsch et al., 2014; Nikolova et al., 2011). Indeed, it was found that the fetus of the mice who were orally exposed to various doses of DEP ranging from 31.25 mg/kg/day to as high as 500 mg/kg/day, had a significant increase in the frequency of DNA deletions (Reliene et al., 2005). Furthermore, the interactions of the low doses of particulate matter with the immune system and their toxicity status have been discussed by different research groups (Ernst et al., 2002; Miyata and van Eeden, 2011). In accordance with these evidences, the main goal of this work was to evaluate the immunological consequences of high dose exposure to VEPs that are collected from the locomotive, diesel, and gasoline powered cars in Russia.

2. Materials and methods

2.1. Samples

The samples were collected from a diesel car (engine volume 2.5 lt) (D), a gasoline car (engine volume 4.3 lt) (G) and a diesel locomotive (2×3060 horsepowers) (L) (engine volume not available). The cars were fueled in the same gas station located in Vladivostok, Russia. The fuels of cars and locomotive were from the same manufacturer. All preparations were done by our methods, as described and patented by Golokhvast et al., previously (Golokhvast et al., 2015a, 2015b). Briefly, to collect the samples, the exhaust gas suspension (EGS) method was used. Exhaust gases were collected via a PVC hose and cooled by passing through water, so that up to 80% particulates retained in deionized water (Golokhvast et al., 2015a, 2015b). The particles sizes were ranging from 1 to 10 μm by volume count, and from 0.1 to 1 μm by number count.

2.2. Experimental animals

SPF-class Kunming mice (male; age, 4 weeks; weight, 15–20 g) were accommodated one week before experiments. They kept in a room, in plastic cages at room temperature 22–27 °C, relative humidity 55 ± 15% and 12 h dark/light cycle. Mice received a balanced diet and water unlimited. Male mice aged 5 weeks and weighing 20–25 g were used for all further experiments. The animals were randomly assigned to the groups. The study protocol was reviewed and approved by the Animal Care Committee of Far Eastern Federal University. The study followed guiding principles for experimental procedures of Declaration of Helsinki for animal experimentation.

2.3.1. VEP sample analysis

The methanol and water soluble fractions of VEP were analyzed. To extract the methanol soluble fraction, 10 mg of each sample was diluted in 1 ml of methanol and placed in an ultrasonic water bath for 10 min. After vortexing for 20 s, the supernatant was centrifuged at 14,000 rpm for 5 min and analyzed by Gas chromatography-Mass spectrometry (GC-MS).

In order to determine the water soluble contents, moreover, 4–20 mg of each sample was placed in Solid Phase Microextraction vials containing 1 ml of ultrapure water, 200 mg of NaCl and sealed with Polytetrafluoroethylene/silicon septum caps. Online extraction was performed with a 65 μm Polydimethylsiloxane/Divinylbenzene Metal Alloy type fiber, at 90 °C for 20 min with an agitation speed at 250 rpm. After the absorption of the analytes was complete, the fiber tip was inserted in the injection port of the GC-MS for 3 min

2.3.2. Instrumentation

Analysis was carried out by a GC-MS instrument (Shimadzu QP-2010) equipped with a split/splitless injection inlet and an AOC-5000 auto-sampler. Pure helium (99.999%) was used as flow gas (1 ml/min). The separation of the analytes was achieved by a Supelco Analytical SLBtm-5 ms capillary column of 30 m length, 0.25 mm i.d., 0.25 μm film thickness with initially temperature at 120 °C (stable for 3 min), increased to 310 °C with a rate of 5 °C/min (stable for 1 min) and finally raised to 325 °C (at 10 °C/min, stable for 1 min). The mass spectrometer detector was operated at full scan for screening of the sample using the GC-MS libraries (NIST107.lib, Wiley7.lib) and at the selected ion-monitoring mode for polycyclic aromatic hydrocarbons (PAHs) monitoring and quantification. The inlet temperature, the interface and the ion source temperatures were 300 °C, 310 °C and 230 °C, respectively (Tzatzarakis et al., 2014). The retention times, as well as the used m/z ions for the determination and qualification of PAHs were presented in Table 1.

2.4. In vivo toxicity testing

2.4.1. Survival experiments

The VEPs, at various dose levels were evaluated for their effect on the mice survival rate. Samples of VEPs were sterilized in bactericidal UV box (Liston-U2103, Russia) and then suspended in sterilized saline

Table 1
Retention times and m/z ions used to determine polycyclic aromatic hydrocarbons and internal standard (TCN-IS) by gas chromatography-mass spectrometry.

compound	Rt (min)	Q1 m/z	Q2 m/z
acenaphtylene	10.03	152	76
fluorene	12.96	166	82
anthracene +phenanthrene	17.27	178	76
TCN (IS)	20.77	266	194
pyrene	23.85	202	101
benzo(a)anthracene +chrysene	29.61	228	114
benzo(k)fluoranthene+benzo(a)fluoranthene +benzo(a) pyrene	34.41	252	126
benzo(g,*h*, i)perylene+dibenz(a,h)anthracene	40.18	276	138
indeno(1,2,3-cd)pyrene	40.97	276	138