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Airborne endotoxin in fine particulate matter in Beijing

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HIGHLIGHTS

- We provide the first 1-year continuous airborne endotoxin concentrations in China.
- Large variations in endotoxin concentration were observed.
- Ambient environmental factors were found to influence endotoxin concentrations.
- Chemical compositions of particles were associated with endotoxin concentrations.
- Endotoxin concentration was positively correlated with ROS, but not DTT.

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ABSTRACT

Endotoxin is an important biological component of particulate matter (PM) which, upon inhalation, can induce adverse health effects, and also possibly complicate the diseases in combination with other pollutants. From 1 March 2012 to 27 February 2013 we collected air samples using quartz filters daily for the quantification of airborne endotoxin and also fine PM ($PM_{2.5}$) in Beijing, China. The geometric means for endotoxin concentration and the fraction of endotoxin in PM were 0.65 EU/m^3 (range: 0.10–75.02) and 10.25 EU/mg $PM_{2.5}$ (range: 0.38–1627.29), respectively. The endotoxin concentrations were shown to vary greatly with seasons, typically with high values in the spring and winter seasons. Temperature and relative humidity, as well as concentrations of sulfur dioxide and nitrogen oxides were found to be significantly correlated with airborne endotoxin concentrations ($p < 0.05$). Additionally, positive correlations were also detected between endotoxin concentrations and natural sources of Na^+, K^+, Mg^{2+}, and F^-, while negative correlations were observed between endotoxin concentrations and anthropogenic sources of P, Co, Zn, As, and Tl. Oxidative potential analysis revealed that endotoxin concentrations were positively correlated with reactive oxygen species (ROS), but not dithiothreitol (DTT) of PM. This study provided the first continuous time series of airborne endotoxin concentrations in Beijing, and identifies its potential associations with atmospheric factors. The information developed here can assist in the assessment of health effects of air pollution in Beijing.

1. Introduction

It is well established that exposure to airborne pollutants, especially fine particulate matter (PM) with an aerodynamic diameter of <2.5 μm ($PM_{2.5}$), is associated with adverse health effects (Pope and Dockery, 2006; Brook et al., 2010). The potential of inhaled PM, suggested to elicit a respiratory and/or systemic inflammatory response, is a function of its oxidative potential (Dominici et al., 2006; Brook et al., 2010), and the potential is described as the capability of PM to generate reactive oxygen species (ROS) directly or indirectly, which subsequently leads to oxidative injury (Prahalad et al., 1999; Huang et al., 2012; Strak et al., 2012). Many studies have investigated the association between adverse health effects and the toxicity of the various chemical components of PM (Brunekreef, 2010), and among others the role that transition metals (Steenhof et al., 2011; Li et al., 2012; Shang et al., 2013a) and organic species (polycyclic aromatic hydrocarbons and quinones) (Deng et al., 2006; Wang et al., 2011; Shang et al., 2012) were found to be associated with increased PM toxicity. However, despite being an important biological component of PM, endotoxin is less studied (Allen et al., 2011).

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Endotoxin, chemically defined as lipopolysaccharide (LPS), is ubiquitous in the environment and is an important structural component of the outer membrane of Gram-negative bacteria (Beutler and Rietschel, 2003). Exposure to endotoxin was found to cause and exacerbate asthma and wheezing in both children and adults (Boehlecke et al., 2003; Bolte et al., 2003; Thorne et al., 2005; Abbing-Karahagopian et al., 2012). Endotoxin is also implicated in the impairment of lung function (Rabinovitch et al., 2005; Liebers et al., 2008) and the pathogenesis of pulmonary diseases (Schwartz, 2001; Loh et al., 2006), such as organic dust lung diseases (Rylander, 2006), chronic obstructive pulmonary diseases (COPD) (Schwartz et al., 1995), and acute lung injury (Thorn, 2001). Studies with healthy human subjects have shown an acute dose-related inflammatory response to inhaled LPS, with a rise in the blood cytokine concentrations, such as neutrophils, tumor necrosis factor-alpha, and interleukin-6 (Michel et al., 1997; Nightingale et al., 1998; Alexis et al., 2004; Doyen et al., 2012; Möller et al., 2012). As an important airborne pollutant, airborne endotoxins are shown to be significantly associated with fine PM (Carty et al., 2003; Mueller-Anneling et al., 2004; Tager et al., 2010) and are deposited in the lungs following inhalation (Monn and Koren, 1999). Many investigations reported the contribution of endotoxin to PM toxicity both *in vitro* (Soukup and Becker, 2001; Guastadisegni et al., 2010; Shang et al., 2013a) and *in vivo* (Alexis et al., 2006), even with a mild exposure (Schins et al., 2004; Alexis et al., 2004). Furthermore, studies have found that the inhalation of endotoxins, in combination with other airborne agents such as PM, fungi, allergens, and ozone (O_3), increases the susceptibility to and severity of an immune response, and can lead to other adverse health effects (Takano et al., 2002; Osornio-Vargas et al., 2003; Ryan et al., 2009; Degobbi and Hila, 2011). Exposure to endotoxin will prime targeted cells and enhance the inflammatory response to secondary stimulus from other pollutants (Alexis et al., 2004). However, despite of its important health effects only a few studies have reported airborne concentrations of endotoxin (Mueller-Anneling et al., 2004; Nilsson et al., 2011; Cheng et al., 2012), especially with respect to continuous observations over the course of an entire year (Tager et al., 2010), particularly in densely populated and polluted urban areas.

Beijing is one of the world's megacities, with a population of over 20 million, which frequently experiences poor air quality. A number of studies have investigated the adverse health effects associated with exposure to ambient pollutants in Beijing (Li et al., 2011; Lin et al., 2011; Huang et al., 2012; Rich et al., 2012; Li et al., 2013; Shang et al., 2013b), estimation of exposure of endotoxin is significantly lacking. Here, we studied temporal variations in endotoxin concentrations with meteorological parameters, gaseous pollutants, $PM_{2.5}$ and its chemical components from 1 March 2012 to 27 February 2013. Among others, we also examined the association between endotoxin concentrations and the oxidative potential of PM. This work is the first ever of its kind to report daily concentrations of endotoxin in ambient $PM_{2.5}$ in Beijing, China over the course of an entire year.

2. Methods

2.1. Sample collection

PM samples were collected at an urban monitoring station, which is located on the roof of a six-story building (~30 m above the ground), to monitor air pollutants at a relative large scale, on the campus of Peking University in northwestern Beijing (39°59′21″ N, 116°18′25″ E). The site has been used for monitoring airborne pollutants in numerous studies (Wu et al., 2007; Xie et al., 2008; Yue et al., 2011; Fang et al., 2012). There are two major city express roads located near the station, 150 m to the east and 200 m to the south. No obvious biological sources of endotoxin (e.g., broadleaf trees, sewages) were found near the sampling site. In this study, the site was selected to be representative of regional air pollution sources and not local sources. A total of 321 quartz filter samples and 18 blank filters were collected from 1 March 2012 to 27 February 2013. During this period, there were several days on which no samples were collected due to rain or sampler failures. Continuous air pollutant concentrations and meteorological parameters were also simultaneously measured at the monitoring station. Daily averages of gaseous pollutant concentrations and meteorological variables were calculated for the time period during which daily filter samples were collected. In addition, rainfall data were also obtained from Beijing Meteorological Station. Other details of the sampling site and instrumentations are provided in Supplementary Materials.

2.2. Endotoxin analysis

We extracted endotoxin from filter samples following the procedures reported in previous studies (Mueller-Anneling et al., 2004; Spaan et al., 2008; Tager et al., 2010), and endotoxin concentration was measured using a kinetic chromogenic *Limulus* Amoebocyte Lysate (LAL) assay kit (Associates of Cape Cod, Inc., USA) following the instructions suggested by the manufacturer. Details are provided in Supplementary Materials. Concentrations are reported as endotoxin units (EU). Typically, the detection limit for endotoxin is 0.005 EU/mL; however this could vary with samples and experiments. In another word, the detection limit was the smallest value obtained for a sample that is significantly higher than the negative control for a particular experiment.

In our work, we have conducted sample spikes and negative controls along with the samples in triplicate. All 321 air samples were observed to exceed the detection limit (0.005 EU/mL) in reference to the negative control. All the 18 blank filters were also processed and analyzed, following the same procedures, as negative controls for sample calibration, which were generally at or below the analytical detection limits. For the spiked samples, we found the recovery rate in the range of 50–200% as suggested acceptable by the LAL assay kit. The CV for the replicates in the same microplate was generally less than 10%.

2.3. Other measurements

Various assays have been used to assess the oxidative potential of PM, including the ability of PM to induce hydroxyl radicals (•OH; Li et al., 2012; Shang et al., 2009, 2012), the ability of PM to deplete antioxidants (Mudway et al., 2004), and the promotion of electron transfer measured by the consumption of dithiothreitol (DTT; Biswas et al., 2009; Cho et al., 2005); there is no consensus on which assay is the most appropriate (Ayres et al., 2008). In this study, we chose to use both DTT and reactive oxygen species (ROS) to assess the oxidative potential of PM. In addition, trace elements (Al, Fe, Na, Mg, K, Ca, Ba, Ti, Mn, Co, Ni, Cu, Zn, Mo, Cd, Sn, Sr, Sb, Pb, Tl, Se, Ge, Cs, Ga, V, Cr, As, Se, Rb) and water-soluble ions (Na^+, NH_4^+, K^+, Mg^{2+}, Ca^{2+}, F^-, Cl^-, SO_4^{2-}, NO_3^-) in $PM_{2.5}$ were also analyzed, with inductively coupled plasma–mass spectrometry and ion-chromatograph respectively. Details can be found in the Supplementary Materials. In this work, all collected filter samples ($n = 321$) were analyzed for PM mass and endotoxin concentration, while samples collected on every sixth day ($n = 60$) were subjected to chemical and oxidative potential analyses.