Environmental Pollution 240 (2018) 817–830

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Inhalational exposure to particulate matter air pollution alters the composition of the gut microbiome

Ece A. Mutlu [a, *], Işın Y. Comba [a], Takugo Cho [b], Phillip A. Engen [a], Cemal Yazıcı [c], Saul Soberanes [d], Robert B. Hamanaka [b], Recep Niğdelioğlu [b], Angelo Y. Meliton [b], Andrew J. Ghio [e], G.R. Scott Budinger [d], Gökhan M. Mutlu [b]

[a] Division of Digestive Diseases, Hepatology and Nutrition, Department of Internal Medicine, Rush University Medical Center, Chicago, IL, 60612, USA
[b] Section of Pulmonary and Critical Care Medicine, The University of Chicago, Chicago, IL, 60637, USA
[c] Division of Gastroenterology and Hepatology, University of Illinois at Chicago, Chicago, IL, 60612, USA
[d] Division of Pulmonary and Critical Care Medicine, Northwestern University, Chicago, IL, 60611, USA
[e] United States Environmental Protection Agency, Chapel Hill, NC, 27599, USA

ARTICLE INFO

Article history:
Received 5 January 2018
Received in revised form
11 April 2018
Accepted 27 April 2018

Keywords:
Air pollution
Microbiota
Gastrointestinal
Intestine
Feces

ABSTRACT

Recent studies suggest an association between particulate matter (PM) air pollution and gastrointestinal (GI) disease. In addition to direct deposition, PM can be indirectly deposited in oropharynx via muco-ciliary clearance and upon swallowing of saliva and mucus. Within the GI tract, PM may alter the GI epithelium and gut microbiome. Our goal was to determine the effect of PM on gut microbiota in a murine model of PM exposure via inhalation. C57BL/6 mice were exposed via inhalation to either concentrated ambient particles or filtered air for 8-h per day, 5-days a week, for a total of 3-weeks. At exposure's end, GI tract tissues and feces were harvested, and gut microbiota was analyzed. Alpha-diversity was modestly altered with increased richness in PM-exposed mice compared to air-exposed mice in some parts of the GI tract. Most importantly, PM-induced alterations in the microbiota were very apparent in beta-diversity comparisons throughout the GI tract and appeared to increase from the proximal to distal parts. Changes in some genera suggest that distinct bacteria may have the capacity to bloom with PM exposure. Exposure to PM alters the microbiota throughout the GI tract which maybe a potential mechanism that explains PM induced inflammation in the GI tract.

1. Introduction

Particulate Matter (PM) is a component of air pollution and has been linked with cardiovascular diseases (Brook et al., 2010; Dai et al., 2014), lung cancer (Hamra et al., 2014), impairment of lung development and a decrease in lung function (Paulin and Hansel, 2016), community acquired pneumonia (Neupane et al., 2010b), deep vein thrombosis (Baccarelli et al., 2008), and lower verbal learning performance (Gatto et al., 2014). Recent studies also reveal a link between PM and gastrointestinal (GI) disease including appendicitis (Kaplan et al., 2009), colorectal cancer (Lopez-Abente et al., 2012) and increased hospitalization of patients with inflammatory bowel disease (IBD) (Ananthakrishnan et al., 2011). These findings strongly suggest an association between PM exposure and inflammatory diseases of GI tract (Kaplan et al., 2010). World Health Organization has ranked PM related air pollution as the 13th most common cause of overall mortality in the world and attributed over 3 million premature deaths per year to outdoor pollution in 2012 (World Health Organization 2014, 2016). The impact of air pollution on human mortality has recently been confirmed by another study showing that PM air pollution, leads to 3.3 million premature deaths per year worldwide (Lelieveld et al., 2015). These findings underscore the magnitude of the health effects that PM exposure may potentially cause (World Health Organization 2014, 2016).

* Corresponding author. Rush University Medical Center, Section of Gastroenterology, Hepatology and Nutrition, 1725 W Harrison, Suite 206, Chicago, IL, 60612, USA.

E-mail addresses: Ece_Mutlu@rush.edu (E.A. Mutlu), yagmurcomba@gmail.com (I.Y. Comba), jared.cho@gmail.com (T. Cho), Phillip_A_Engen@rush.edu (P.A. Engen), cemalyazici@yahoo.com (C. Yazıcı), saulaxo@gmail.com (S. Soberanes), rhamanaka@medicine.bsd.uchicago.edu (R.B. Hamanaka), recep@uchicago.edu (R. Niğdelioğlu), ameliton@medicine.bsd.uchicago.edu (A.Y. Meliton), Ghio.Andy@epa.gov (A.J. Ghio), s-buding@northwestern.edu (G.R.S. Budinger), gmutlu@medicine.bsd.uchicago.edu (G.M. Mutlu).

https://doi.org/10.1016/j.envpol.2018.04.130
0269-7491/

After initial inhalation, where the inhaled particles are deposited depends on their size. Most of the larger particles are sequestered in the upper airway or in the conducting lower airways such as the trachea and larger bronchi (Kreyling et al., 1999; Moller et al., 2004; Oberdorster, 1993). Smaller size particles, particularly those that are less than 2.5 microns in mean diameter ($PM_{2.5}$) can reach the bronchioles and alveolar spaces, where they are phagocytosed by alveolar macrophages (Kreyling et al., 1999; Moller et al., 2004; Oberdorster, 1993). Particles sequestered in macrophages and directly in the mucus layer in the lower airways are subsequently transported up to the oropharynx and then swallowed into the GI tract (Beamish et al., 2011; Oberdorster, 1993; Semmler-Behnke et al., 2007). Furthermore, PM can also be ingested directly by consumption of food and water contaminated by PM (Beamish et al., 2011; Commission, 2002; De Brouwere et al., 2012; Kampa and Castanas, 2008; Oberdorster, 1993; Salim et al., 2014b). It has been estimated that 10^{12}-10^{14} particles are ingested per day by an individual on a typical Western diet (Lomer et al., 2002, 2004). Collectively, the GI tract can be exposed to significant amounts of PM through these direct and indirect routes of exposure (Beamish et al., 2011; De Brouwere et al., 2012; Oberdorster, 1993; Salim et al., 2014b).

We have previously examined the effect of PM on GI permeability and pro-inflammatory cytokine production (Mutlu et al., 2011). In that study, we demonstrated that exposure to PM increased gut permeability both in cell-based and animal models. Treatment of gut epithelial cells with PM caused increased production of mitochondrial reactive oxygen species (ROS), release of inflammatory cytokines and induced apoptosis of colonocytes (Mutlu et al., 2011). While our murine model confirmed the effect of PM that we observed on enterocytes *in vitro*, the translation of these findings to human exposure was limited as we had used a single dose instillation of PM via gastric lavage to evaluate the effects of PM on GI tract.

The unwanted health effects of PM on the GI tract may not be limited to its effects on the GI epithelium. When PM enters the GI tract, it not only gets in contact with the GI epithelial and immune cells, but also with more than 10^{14} microbes residing there. Growing evidence suggest that alterations in the composition and diversity and function of gut microbiota may play a role in the development of GI diseases such as IBD as well as other inflammatory disorders of the GI tract.

In this study, we aimed to determine whether exposure to inhaled PM at clinically relevant doses alters the bacterial composition throughout the gastrointestinal tract in mice. This is the first study that investigated the effects of PM on microbiome composition of GI tract using a clinically relevant model of PM exposure via inhalation.

2. Materials and methods

2.1. Animals

The research protocol was evaluated and approved by the Animal Care and Use Committee of Northwestern University, and the University of Chicago in Chicago, Illinois. The mice were 20–25 g, male, 8–12 weeks old and these C57BL/6 mice were obtained from Jackson Laboratories. Ten mice were allocated into each study group. Mice received the 2918 Teklad global 18% protein rodent diet (Envigo, Indianapolis, IN) prior to exposure and during the times when they were not being exposed to PM or filtered air in the exposure chambers. During the 8-h exposure to PM or FA, they received Diet Gel 76A (ClearH2O, Westbrook, ME).

2.2. Inhalational exposure to $PM_{2.5}$

We exposed mice to $PM_{2.5}$ concentrated from ambient air in Chicago 8 h per day for 5 days a week for three consecutive weeks in a chamber connected to a Versatile Aerosol Concentration Enrichment System (VACES) (Budinger et al., 2011; Chiarella et al., 2014). The VACES system draws approximately 100 L per minute of ambient air from which PM is condensed and then resuspended for delivery to a chamber designed specifically to ensure uniform distribution of the particles. We exposed control mice to filtered air in an identical chamber connected to the VACES in which a Teflon filter was placed on the inlet valve to remove all particles. We estimated ambient $PM_{2.5}$ concentrations as the mean of reported values from the 4 EPA monitoring locations closest to our location (State of Illinois Environmental Protection Agency, 2014). Particle counts in the chamber were measured with a TSI 3775 particle counter (Shoreview) and used to determine the enrichment in the chamber compared with the ambient air as previously described (Budinger et al., 2011; Chiarella et al., 2014). The mean daily ambient $PM_{2.5}$ concentration in Chicago was $16.3 \pm 0.85\ \mu g/m^3$ during the study period, and the mean concentration in the PM exposure chamber was $135.4 \pm 6.4\ \mu g/m^3$. Chicago is the third largest city in the US, with approximately 2.7 million and 9.5 million residents in the city and metropolitan area, respectively. Interstate highways, railroads, and 2 major airports connect the city to other urban areas in the region. Major point sources of particulate air pollution include 2 coal-fired power plants and metal processing, paint, and solvent factories (the last being in the southern and southeast parts of the city) (Binaku et al., 2013). Mobile source emissions account for the majority of atmospheric nitrogen compounds, while refineries, coal burning, and steel manufacturing are responsible for sulfur compounds (Binaku et al., 2013). The composition of airborne PM is primarily sulfate and organic carbons and secondary nitrates. Particulate NO_3^-, SO_4^{2-}, and elemental carbon concentrations (2.5, 2.9, and 1.5 $\mu g/m^3$, respectively) approximate those in other major American cities (Babich et al., 2000).

2.3. Characterization of PM

To determine the chemical composition of $PM_{2.5}$ that our mice were exposed, four blank and four PM-exposed Teflon filters (PTFE, 37 mm, 2 μm pore; PALL Life Sciences, Ann Arbor, MI) with a mean (±SD) particle mass of 1.1 ± 0.5 mg were agitated for 1 h in 3.0 mL 1.0 N HCl. Supernatants were analyzed for metals (in duplicates) using inductively-coupled plasma optical emission spectrometry (ICP-OES; Model Optima 4300D, Perkin Elmer, Norwalk, CT) operated at two separate wavelengths for each metal. Chemical composition of $PM_{2.5}$ is shown in Table 1.

Table 1
Chemical composition of PM.

	Blank filter (mean ± SD)	$PM_{2.5}$ (mean ± SD)
[Ca] (ppm)	0.121 ± 0.040	9.628 ± 4.825
[K] (ppm)	0.021 ± 0.009	1.373 ± 0.151
[Mg] (ppm)	0.005 ± 0.004	2.637 ± 1.460
[Na] (ppm)	0.033 ± 0.018	9.287 ± 0.815
[Cd] (ppm)	BDL	BDL
[Cu] (ppm)	0.0003 ± 0.001	0.202 ± 0.058
[Fe] (ppm)	0.072 ± 0.044	3.878 ± 1.086
[Mn] (ppm)	0	0.134 ± 0.014
[Pb] (ppm)	0.0035 ± 0.001	0.666 ± 0.423
[Zn] (ppm)	0.0110 ± 0.003	1.211 ± 0.217

BDL, below detection limit.