Respiratory Physiology & Neurobiology 243 (2017) 39–46

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

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

The effect of exhalation flow on endogenous particle emission and phospholipid composition

Per Larsson[a,*], Björn Bake[b], Anita Wallin[a], Oscar Hammar[c], Ann-Charlotte Almstrand[a], Mona Lärstad[a], Evert Ljungström[d], Ekaterina Mirgorodskaya[a,1], Anna-Carin Olin[a,1]

[a] Unit of Occupational and Environmental Medicine, Department of Public Health and Community Medicine, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden
[b] Unit of Respiratory Medicine and Allergy, Department of Internal Medicine, Institute of medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden
[c] AstraZeneca, R & D, Pepparedsleden 1, 431 50 Mölndal, Sweden
[d] Atmospheric Science, Department of Chemistry and Molecular Biology, University of Gothenburg, Gothenburg, Sweden

ARTICLE INFO

Keywords:
PEx
Exhaled particles
Pulmonary surfactant
Non-invasive technique
Forced expiration
Airway re-opening

ABSTRACT

Exhaled particles constitute a micro-sample of respiratory tract lining fluid. Inhalations from low lung volumes generate particles in small airways by the airway re-opening mechanism. Forced exhalations are assumed to generate particles in central airways by mechanisms associated with high air velocities. To increase knowledge on how and where particles are formed, different breathing manoeuvres were compared in 11 healthy volunteers. Particles in the 0.41–4.55 µm diameter range were characterised and sampled. The surfactant lipid dipalmitoylphosphatidylcholine (DPPC) was quantified by mass spectrometry.

The mass of exhaled particles increased by 150% (95% CI 10–470) for the forced exhalation and by 470% (95% CI 150–1190) for the airway re-opening manoeuvre, compared to slow exhalations. DPPC weight percent concentration (wt%) in particles was 2.8 wt% (95%CI 1.4–4.2) and 9.4 wt% (95%CI 8.0–10.8) for the forced and the airway re-opening manoeuvres, respectively.

In conclusion, forced exhalation and airway re-opening manoeuvres generate particles from different airway regions having different DPPC concentration.

1. Introduction

Non-invasive methods capable of selective sampling of respiratory tract lining fluid (RTLF) from different airway regions are of great interest for clinical research. We have developed a method for counting and collection of exhaled particles, in the 0.4–4.6 µm diameter range, that enables quantitative analysis of small airway RTLF composition (Almstrand et al., 2009; Larsson et al., 2012). This method has been used to identify changes in RTLF protein content among COPD patients (Larstad et al., 2015) and to detect alterations in protein composition associated with bronchiolitis obliterans syndrome in lung transplant recipients (Ericson et al., 2016). For reproducible sampling, it is important to understand how particle emission and the chemical composition of exhaled particles are influenced by different breathing parameters. Exhaled particles from breathing manoeuvres that induce airway closure and re-opening showed lipid (Almstrand et al., 2012) and protein (Bredberg et al., 2012) composition similar to that observed in bronchoalveolar lavage (BAL) fluid. This is consistent with the hypothesis of particle formation in small airways as a result of RTLF film rupture during inspiration from very low lung volumes (Almstrand et al., 2010; Haslbeck et al., 2010; Holmgren et al., 2010; Johnson and Morawska, 2009). The absence of any mucins in particles produced by the airway closure and re-opening manoeuvre supports a peripheral formation site of these particles (Bredberg et al., 2012).

In parallel with exploration of RTLF samples from the lung periphery, it is of interest to study RTLF samples from more central airways. During forced exhalations and cough, respiratory muscles compress the lungs, which causes the airways to compress dynamically (Dawson and Elliott, 1977). The dynamic airway compression results in high linear airflow velocities, producing shear forces (Moriarty and Grotberg, 1999) and airway wall flutter (Bertram and Elliott, 2003; Grotberg and Shee, 1985), that almost certainly would generate particles in more central airways than the terminal bronchiols. There are a few reports on exhaled particle concentrations and size distribution during speaking, vocalization and cough (Chao et al., 2009; Johnson et al., 2011; Papineni and Rosenthal, 1997; Yang et al.,

* Corresponding author at: Sahlgrenska Academy, University of Gothenburg, Box 414, 405 30 Gothenburg, Sweden.
E-mail address: per.larsson@amm.gu.se (P. Larsson).
[1] Last authorship is shared between Ekaterina Mirgorodskaya and Anna-Carin Olin.

http://dx.doi.org/10.1016/j.resp.2017.05.003
Received 27 January 2017; Received in revised form 5 May 2017; Accepted 10 May 2017
Available online 11 May 2017

2007). In these studies, particle concentrations and size distributions were studied and compared to theoretical models of particle formation and deposition but no chemical analysis of the particles was performed to confirm the results.

The phospholipid dipalmitoylphosphatidylcholine (DPPC), also known as PC(16:0/16:0), is the major component of surfactant and constitutes between 40 and 60 mole% (Postle et al., 2001; Wright et al., 2000; Zuo et al., 2008) of the phosphatidylcholines. DPPC is produced by the alveolar type II pneumocytes and modulates surface tension to prevent airway collapse. It is not known to be produced by other cells in the airways (Bernhard et al., 1997); however, surfactant is likely transported towards the glottis by mucociliary transport. The presence of DPPC in tracheal aspirates from animals (rat and porcine) (Bernhard et al., 1997; Rau et al., 2003) and humans (Dushianthan et al., 2014) suggests that DPPC may be present all the way from the alveolus to glottis. The concentration of DPPC in RTLF is likely to decline with the distance from the alveoli due to dilution, uptake and degradation. Palmitoyl-oleoyl-phosphatidylcholine (POPC), also known as PC (16:0/18:1), constitutes around 10 mole% (Bernhard et al., 2004) of the phosphatidylcholines in human BAL and is the most abundant of the unsaturated phosphatidylcholines. Unlike DPPC, POPC is a common component in cell membranes and lower DPPC to POPC ratio was reported in airway mucosa compared to that in lung parenchyma and BAL (Bernhard et al., 1997).

In the present study we hypothesize that different breathing manoeuvres can be used to sample particles from different airway regions. The specific aims were to study how maximally forced exhalations influence particle formation compared to slower exhalations and to compare the DPPC and POPC concentration in particles exhaled by maximal forced exhalation to those produced in small airways during airway re-opening.

2. Materials and methods

2.1. Subjects

A total of 6 men and 5 women, aged 28–75 years and recruited from our department, were included in the study. They had no respiratory illness to report and their lung function was normal, as examined by spirometry (SPIRARE SPS310 sensor and SPIRARE 3 software, Diagnostica AS, Oslo, Norway) performed in accordance with the ATS/ERS criteria (Miller et al., 2005). Predicted values were calculated using the ECCS/ERS reference equations (Quanjer et al., 1993). The research was conducted according to the principles of the Declaration of Helsinki. All participants gave their written informed consent, and the study protocol was approved by the Ethical Review Board of the Medical Faculty at the University of Gothenburg, Sweden.

2.2. Study design

Particle formation induced by airflow and airway re-opening mechanisms was studied by using specific breathing manoeuvres.

The breathing manoeuvres were:

i *Reference* (Fig. 1a): Inhalation starts at functional residual capacity (FRC) with 5 s breath hold at total lung capacity (TLC) before a “slow” exhalation to residual volume (RV).
ii *Forced exhalation* (Fig. 1b): The same manoeuvre as the reference with the only exception of a maximal forced exhalation from TLC to RV. This manoeuvre was designed to induce particle formation during exhalation by the air-RTLF interaction at high air velocities.
iii *Cough* (Fig. 1c): The subjects were instructed to inhale to TLC and perform a Valsalva manoeuvre against a closed glottis, before performing 2–3 forceful coughs, resulting in an end-respiratory volume between RV and FRC.
iv *Airway re-opening* (Fig. 1d): Deep exhalation to RV, 3s breath hold at RV before inhalation to TLC and a slow exhalation from TLC to RV. This manoeuvre was designed to maximise airway closure and re-opening and induce particle formation during inhalation from RV to TLC.

The reference manoeuvre was designed to be a baseline value for particle formation. These particles constitute a background particle level of particles that is formed during all manoeuvres. For this reason, particle formation by the other manoeuvres is reported as the increase from the reference manoeuvre. The forced exhalation manoeuvre was intended to induce particles by mechanisms associated to high air velocities and the airway re-opening manoeuvre was intended to induce particles formed by the airway opening mechanism. A cough manoeuvre was included since it is known to generate a high amount of particles. It is not known how particles are formed during cough but similar mechanisms as for forced exhalations have been suggested, i.e the air-RTLF interaction at high air velocities.

A breath-hold at total lung capacity was introduced for all manoeuvres except the airway re-opening manoeuvre to minimize the background level of particles formed during the inhalation from FRC to TLC. During breath hold at TLC, a fraction of the particles formed during the inhalation deposit in the airways before the start of the exhalation (Holmgren et al., 2013).

Out of the studied manoeuvres, the airway re-opening manoeuvre was expected to generate particles at the most distal airway region and to have the highest concentration of DPPC. For this reason, the DPPC concentration in exhaled particles were compared to the airway re-opening manoeuvre, where a decreased concentration was considered to indicate a less distal airway origin.

Each subject performed ten repetitions of each breathing manoeuvre. The four different manoeuvres were executed during the same day and the order of the four different manoeuvres where randomized between subjects. All participants wore a nose clip throughout the entire procedure and inhaled air through a high-efficiency particle arresting (HEPA) filter. Before starting the measurement, the participants breathed tidally for 2 min to vent ambient particles from the airways. The exhaled particles were characterized and sampled using the PExA instrument.

2.3. Sampling and chemical analysis

The PExA instrument and method have been described previously by Almstrand et al. (2009). For the purpose of the present study that used forced exhalations, the mouthpiece of the instrument was modified to reduce the backpressure. It was verified that flow volume curves with exhalations into the PExA instrument looked identical to flow volume curves from spirometry. The modified instrument is depicted in the supplementary material (Fig. S1). Particles in the diameter size interval of 0.41–4.55 μm, that are measured and sampled by the PExA instrument, are referred to as PEx, (Particles Exhaled). Particles in exhaled air are collected using a two stage inertial impactor with 50% cut off diameters of 7.0 μm for the first stage and 0.5 μm for the second stage. Particles between 0.5–7.0 μm were sampled onto a thin membrane of hydrophilic polytetrafluoroethylene (FHLC02500, Millipore, Billerica, MA, USA) placed on the second impaction stage. The method used for calculating exhaled and sampled particle mass from measured particle number concentrations has been described previously (Larsson et al., 2015).

Chemical analysis was performed on a triple quadrupole mass spectrometer equipped with an electrospray ionization ion source that operated in positive mode. Quantification of DPPC and POPC were performed with a selected reaction monitoring method using the transitions m/z 734.6 > 184.1 and 760.6 > 184.1 respectively. As internal standards, PC (17:0–14:1) and PC (17:0–20:4) were used for DPPC and POPC quantification, respectively (LM-1002 and LM-1004, Avanti lipids Alabaster, AL, USA). The mass transitions for PC