Title

Lipidomics Profiling of Inflammatory and Specialized Pro-resolving Mediators at the Junction of Inflammation-Resolution Transition in Mouse Lungs Exposed to Multiwalled Carbon Nanotubes

Introduction

Multi-walled carbon nanotubes (MWCNTs) are new nanomaterials made of graphene sheets that role into multi-layer, concentric tubes. MWCNTs are airborne and resistant to natural and biological degradation. The potential that inhalation of MWCNTS at the workplace overtime causes lung injury and disease is a considerable health concern for exposed workers. Exposure to certain MWCNTs induces prominent acute inflammation with lung injury in laboratory animals. How fibrogenic MWCNTs instigate inflammation and how the interplay between inflammation and resolution of inflammation influences fibrotic development from exposure to MWCNTs have not been well understood. This study addressed these knowledge gaps by using a quantitative lipidomics approach to systematically profile inflammatory lipid mediators and specialized pro-resolving lipid mediators, major mediators of inflammation and resolution, respectively, at the junction of transition from acute inflammation to fibrotic progression in mouse lungs exposed to fibrogenic MWCNTs.

Methods Collection

1. **Particle preparation**
   - MWCNTs (Mitsui-7) was prepared in a dispersion medium (DM; 0.9% saline supplemented with 0.6 mg/mL mouse serum albumin, and 0.01 mg/mL 1,2-dipalmitoyl-sn-glycero-3-phosphocholine) following a two-step dispersion procedure.
2. **Animal treatment**
   - Eight-week-old male mice (C57BL/6J) were purchased from the Jackson Laboratories (Bar Harbor, ME, USA). Mice were treated with a single dose of DM or MWCNTs (1860.4 μg/kg body weight) at a volume of 50 μL per mouse by pharyngeal aspiration. Mice were euthanized with an intraperitoneal injection of a sodium pentobarbital euthanasia solution (100-300 mg/kg body weight; Zoetis, Florham Park, NJ, USA), followed by exsanguination once the mouse was unresponsive to toe pinch. Lung tissue was collected, minced, and frozen in liquid nitrogen, and were stored at -80^{o}C for lipidomic and protein analyses.
3. **Immunohistochemistry**
   - Mouse left lungs were formalin-fixed, paraffin-embedded, and sectioned to 5 microns following standard procedures. Immunohistochemistry was performed with the ImmPRESS polymer detection system (Vector Laboratories) using the ImmPACT NovaRED as the peroxidase substrate (red). Nuclei were counterstained with Hematoxylin OS (blue-violet, Vector Laboratories). The primary antibodies used were anti-Mac-2 (Cedarlane, Burlington, Ontario, Canada) and anti-neutrophil (Abcam, Cambridge, MA, USA) antibodies.
4. **Cell culture and fluorescence detection of phospholipids**

   J774A.1 murine monocyte/macrophage cells phagocytose nanoparticles avidly and secrete copious amounts of proinflammatory cytokines such as interleukin-1β and tumor necrosis factor-α in

response to nanoparticle exposure, features observed for mouse alveolar macrophages. J774A.1 cells were grown in a 24-well plate overnight, treated with vehicle or MWCNTs at 2 μg/mL, and stained with the LipidTox Red Phospholipidosis reagent (LTRP). 4',6-diamidino-2-phenylindole (DAPI, Thermo Fisher Scientific) was used to counter stain the nucleus. Images were taken using the Agilent Cytation 1 Cell Imaging Multi-Mode Reader using phase contrast for optical morphology, a DAPI filter for DAPI fluorescence (EX 360 nm, EM 460 nm), and a TRITC filter for LTRP fluorescence (EX 595 nm, EM 615 nm). Data was analyzed with the Agilent Gen5 software.

5. **Mouse bronchoalveolar lavage (BAL)**
   - Mice were euthanized. A tracheal cannula was inserted into the tracheal and BAL was performed through the cannula using ice cold Ca^{2+}- and Mg^{2+}-free phosphate buffered saline (PBS), pH7.4. BAL fluids collected were stored at -80^{o}C till use.
6. **UPLC-MS/MS analytical method**
   - Liquid chromatography. A Waters liquid chromatography-tandem quadrupole mass spectrometer (Waters ACQUITY Xevo TQ-XS UPLC-MS, Waters, Milford, MA, USA) was utilized for lipidomic analysis. The Acquity Premier UPLC system operates with an ACQUITY Premier column (ACQUITY Premier HSS T3 column with a VanGuard FIT, 1.8 μm, 2.1 x 100 mm, Product number 186009471, Waters) using a 10 μL injection volume. The mobile phase gradient consisted of solvent A (95% Milli-Q ultrapure water, 5% methanol, and 0.01% acetic acid with 10 mM ammonium acetate) and solvent B (95% methanol, 5% Milli-Q ultrapure water, and 0.01% acetic acid with 10 mM ammonium acetate). The flow rate was set to 200 μL/min. The gradient began with 100% solvent A and 0% solvent B, and solvent B was gradually increased to 70% over 17 minutes. Solvent B was then gradually increased to 85% over three minutes from 17 to 20 minutes and to 100% at 25 minutes, where it was held for 2 minutes. For re-equilibration, solvent B was reduced to 0% from 27 to 28 minutes and held for an additional 2.5 minutes. The total run time was 30.5 minutes per cycle.
   - MS parameters and transitions. The tandem quadrupole mass spectrometer (Waters Xevo TQ-XS) was operated with an electrospray ionization (ESI) source in the negative-ionization mode for analysis of target analytes.
7. **UPLC-MS/MS Standards and quantification**
   - Internal standard quantification was performed using TargetLynx and MassLynx V4.2. Calibration standards for the 36 target LMs were prepared at 2 different calibration ranges to accommodate the different levels of endogenous concentrations of target LMs in samples.
8. **Sample preparation and solid phase extraction**
   - Sample preparation. Mouse lung tissue was collected and homogenized in ice-cold methanol spiked with the internal standard mixture with a Dounce homogenizer.
   - Solid phase extraction. Extraction of SPMs was performed using a spin column method.
9. **Immunoblotting**
   - Lung tissues were extracted in a lysis buffer (10 mM Tris, pH 7.4, 1% SDS) containing 1x proteinase inhibitor cocktail (Thermo Fisher Scientific) using tissue homogenizer (Omni International, GA, USA) after sonication for 10 seconds. Supernatants were collected and combined for each group. Proteins of 20 mg were fractionized on 8 or 10% SDS-polyacrylamide gel and blotted on nitrocellulose

membranes using specific antibodies. Protein bands were detected using enhanced chemiluminescence and quantified using ImageJ Software (NIH) with normalization to β-actin.

10. Enzyme-linked immunosorbent assay (ELISA)

- Cytokines were detected from lung tissue extracts using ELISA following instructions from the vendor (MyBioSource, San Diego, CA, USA). Measurement was performed using a multi-mode microplate reader from Agilent (Santa Clara, CA, USA).

Citations: Publications based on the dataset

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