Toxicology Reports 1 (2014) 802–811

Contents lists available at ScienceDirect

Toxicology Reports

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

Emissions from commercial-grade charbroiling meat operations induce oxidative stress and inflammatory responses in human bronchial epithelial cells

Ning Li[a,*], Poulomi Bhattacharya[a], Georgios Karavalakis[b], Keisha Williams[a], Nicholas Gysel[b], Nachamari Rivera-Rios[a]

[a] Department of Pathobiology and Diagnostic Investigation, College of Veterinary Medicine, Michigan State University, East Lansing, MI, USA

[b] Center for Environmental Research and Technology, Bourns College of Engineering, University of California Riverside, Riverside, CA, USA

ARTICLE INFO

Article history:
Received 8 August 2014
Received in revised form
19 September 2014
Accepted 24 September 2014
Available online 2 October 2014

Keywords:
Commercial charbroiling meat emissions
Human bronchial epithelial cells
Inflammatory response
Oxidative stress
p38 MAPK
Environmental and occupational health

ABSTRACT

Commercial charbroiling emissions are a significant source of ambient particulate matter (PM) in urban settings. The objective of this study was to determine whether organic extract of PM emissions from commercial charbroiling meat operations could induce an inflammatory response in human bronchial epithelial cells and whether this effect was mediated by oxidative stress. PM samples were collected during cooking hamburgers on a commercial-grade under-fired charbroiler and sequentially extracted with water and methanol to obtain the aqueous PM suspension (AqPM) and organic extract (OE). The pro-oxidative and pro-inflammatory effects of OE were assessed using human bronchial epithelial cell line BEAS-2B. While AqPM did not have any effect, OE effectively induced the expression of heme oxygennase-1 and cyclooxygenase-2 in BEAS-2B cells. OE also up-regulated the levels of IL-6, IL-8, and prostaglandin E2. OE-induced cellular inflammatory response could be effectively suppressed by the antioxidant N-acetyl cysteine, nuclear factor (erythroid-derived 2)-like 2 activator sulforaphane and p38 MAPK inhibitor SB203580. In conclusion, organic chemicals emitted from commercial charbroiling meat operations could induce an inflammatory response in human bronchial epithelial cells, which was mediated by oxidative stress and p38 MAPK.

1. Introduction

Epidemiological and experimental evidence has established a close association between airborne particulate matter (PM) and increased morbidity and mortality of respiratory and cardiovascular diseases [1–6]. The transport sector is known to be the major source of PM in urban environments that is responsible for air quality deterioration. Another significant source contributing to the organic compound mass of urban PM is meat cooking emissions [7,8]. With the advance in engine and fuel technology traffic-derived PM emissions have been markedly reduced

Abbreviations: AqPM, aqueous PM suspension; COX, cyclooxygenase; DEP, diesel exhaust particles; HO-1, heme oxygenase-1; MAPK, mitogen activated protein kinase; NAC, N-acetyl cysteine; OC, organic carbon; OE, organic extract; PAH, polycyclic aromatic hydrocarbon; PG, prostaglandin; PM, particulate matter; SFN, sulforaphane; SOD2, superoxide dismutase 2; TSLP, thymic stromal lymphopoietin; UFP, ultrafine particles.

* Corresponding author at: Michigan State University, 1129 Farm Ln, B43, East Lansing, MI 48840, USA. Tel.: +1 517 8842075; fax: +1 517 4322391.
E-mail addresses: lining3@msu.edu, lining3@cvm.msu.edu (N. Li).

http://dx.doi.org/10.1016/j.toxrep.2014.09.015

and changed, as opposed to the emissions from commercial cooking operations, which are projected to further increase due to the lack of effective controls and regulations.

PM emissions from commercial cooking contribute significantly to nationwide emission inventories, with under-fired charbroiling cooking operations being the major source of PM emissions compared to other cooking styles [9]. Meat cooking operations have been identified to contribute significantly to urban organic aerosol concentrations. Studies have shown that organic acids (e.g., palmitic acid and oleic acid) and cholesterol are considered as tracers for cooking organic aerosol in urban areas [9]. Meat cooking PM emissions consist of almost entirely organic carbons (OC) including polycyclic aromatic hydrocarbons (PAHs) that are classified as hazardous air pollutants by the US EPA [9,10]. Charbroiling meat also emits saturated and unsaturated fatty acids (e.g., alcanoic and alkenoic acids) and heterocyclic aromatic amines [11,12]. The latter compounds have been identified as potent mutagens and carcinogens in experimental animals and potentially carcinogenic to humans [13]. Moreover, several PAH compounds found in commercial charbroiling emissions, as reported by the National Emission Inventory, are associated with the adverse health effects of well-studied diesel exhaust particles (DEP) [10,14–17]. Thus, the similarities in the organic chemical compositions between commercial charbroiling emissions and DEP suggest that this understudied and unregulated emission source may also have adverse health effects.

PM-associated organic chemicals (e.g., PAHs and quinones) can generate reactive oxygen species by undergoing biotransformation and redox cycling inside cells, which can lead to oxidative stress. A stratified oxidative stress response model has been proposed to explain the mechanisms by which traffic-related PM such as DEP and ambient ultrafine particles (UFP) causes cellular injuries. Supporting experimental evidence has shown that PM with high OC/PAH content can elicit a stratified cellular oxidative stress response including activation of nuclear factor (erythroid-derived 2)-like 2 (Nrf2)-mediated antioxidant defense, inflammation and cytotoxicity [18,19]. Exposure of airway epithelial cells can result in increased production of inflammatory cytokines and chemokines through the activation of MAPK and NFkB signaling pathways [18–20]. It can also lead to the up-regulation of lipid inflammatory mediators via cyclooxygenase-2 (COX-2)-mediated prostaglandin (PG) pathways [21].

While detailed studies on the respiratory effects of commercial-grade charbroiling meat cooking emissions have not been conducted, a health hazard evaluation conducted by National Institute for Occupational Safety and Health (NIOSH) in 2009 reported that respiratory symptoms (e.g. wheezing, nasal allergies, and shortness of breath) were common among commercial kitchen workers [22]. This suggests that prolonged exposures to these emissions may have adverse respiratory effects not only on restaurant workers but also on the residents in nearby communities, especially in densely populated major cities. Based on the stratified oxidative stress response paradigm and the resemblance in the OC/PAH composition between charbroiling meat and traffic-related emissions we sought to determine whether organic chemical components emitted from commercial-grade charbroiling meat operations could induce an inflammatory response in human bronchial epithelial cells and whether this effect was mediated by oxidative stress. We show that exposure to the organic extract of commercial-grade charbroiling meat emissions could induce an inflammatory response in human bronchial epithelial cell line BEAS-2B, a widely used in vitro model for investigating the effects of PM. We also demonstrate that the inflammatory response was mediated by cellular oxidative stress and p38 MAPK.

2. Materials and methods

2.1. Reagents

Cell culture-grade water, phosphate buffered saline (PBS), heat-inactivated fetal bovine serum (FBS), N-acetyl cysteine (NAC) and Bradford protein assay reagents were purchased from Sigma (St. Louis, MO). Bronchial epithelial cell growth medium bullet kit (BEGM) was obtained from Lonza (Walkersville, MD). Dulbecco's Modified Eagle Medium (DMEM), penicillin/streptomycin mix and trypsin-EDTA were from Invitrogen (San Diego, CA). OptEIA™ Human IL-6 and IL-8 ELISA kits and type I rat tail collagen were purchased from BD Bioscience (San Diego, CA). Trypan blue solution and chemiluminescent substrate were from HyClone (Waltham, MA) and Thermo Fisher Scientific (Rockford, IL), respectively. Antibodies against phospho-p38, total p38, COX-2, and cell lysis buffer were obtained from Cell Signaling Technology (Beverly, MA). Monoclonal anti-heme oxygenase-1 (HO-1) Ab and PGE2 ELISA kit were from Enzo Life Sciences (Farmingdale, NY). R,S-Sulforaphane (SFN) and SB203580 were purchased from LKT Laboratories (St. Paul, MN) and EMD Millipore (Bedford, MA), respectively. RNeasy Mini Kit was from Qiagen (Valencia, CA). High Capacity cDNA Reverse Transcription Kit was purchased from Applied Biosystem (Grand Island, NY).

2.2. PM collection, chemical analysis and sample preparation

Meat cooking experiments were conducted at the commercial cooking testing facility of the Center for Environmental Research & Technology at the University of California, Riverside using a protocol that specifies the properties of the meat (i.e. 20% fat by weight, 58–62% moisture content, 5/8-in. thickness, and 5-in. diameter), cooking conditions (i.e. 600 °F grate temperature and load capacity of 12 beef patties on the broiler grate), and cooking procedure (i.e. 4.5 min for the first side and 3 min for the second side). Fig. 1A shows that filters were sampled raw from the primary exhaust duct downstream of the natural gas-fueled under-fired charbroiler with a sampling manifold system. PM with aerodynamic diameter <2.5 μm (PM2.5) were collected on 47-mm Pall Gellman Teflo® filters (Ann Arbor, MI) and stored at −80 °C. Samples for elemental carbon (EC) and organic carbon (OC) analysis