



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

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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 oxygenase-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.

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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.

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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 NFκB 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 (PM_{2.5}) 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

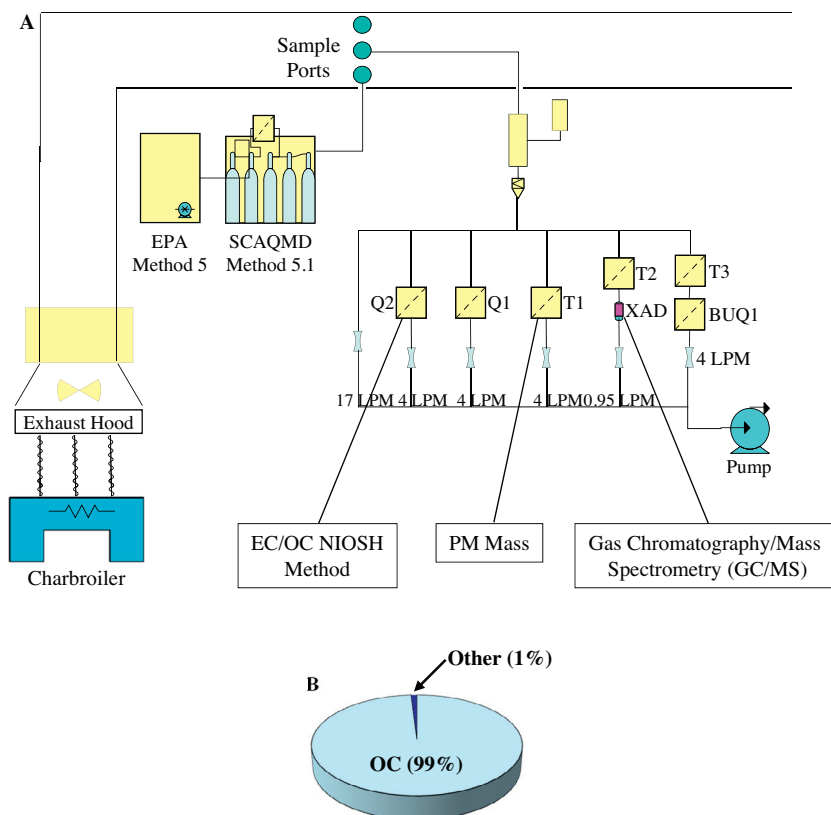


Fig. 1. (A) Configuration of the charbroiled meat cooking and emission collection system in the commercial cooking testing facility. Filters were sampled raw from primary exhaust duct downstream of gas-fueled under-fired charbroiler with a sampling manifold system. PM_{2.5} samples for biological studies were collected on 47 mm Teflon filters. Samples for EC and OC analysis were collected on 47 mm quartz filters. (B) Chemical analysis showing that commercial-grade charbroiling meat emissions contained almost exclusively organic carbons.

were collected on 47-mm 2500 QAT-UP Tissuquartz Pall filters (Ann Arbor, MI), which were preconditioned at 600 °C for 5 h. A Thermal/Optical Carbon Aerosol Analyzer (Sunset Laboratory, Forest Grove, OR) operating on the NIOSH Method 5040 was used to analyze OC and EC (<http://www.cdc.gov/niosh/docs/2003-154/pdfs/5040.pdf>).

Prior to extraction, the filters were brought to room temperature to allow the moisture to evaporate. The filters were extracted with sterile cell culture-grade water to obtain the aqueous PM_{2.5} suspension (AqPM). The filters were removed from AqPM, dried and extracted again with 100% methanol. After methanol evaporation by nitrogen gas the organic extract (OE) was prepared by dissolving extracted materials in DMSO. Aliquots of AqPM and OE were stored at −80 °C. The mass of extracted PM_{2.5} and organic materials was calculated as the difference in filter weight before and after the extraction [23,24]. DEP was a generous gift from Dr. Yoshito Kumagai at University of Tsukuba, Japan [25]. DEP suspension was prepared in PBS as previously described [24].

2.3. BEAS-2B cell culture and determination of cellular viability

Human bronchial epithelial cell line (BEAS-2B) was obtained from American Type Culture Collection (ATCC).

BEAS-2B cells were maintained in BEGM in collagen-coated cell culture plates. To determine the toxicity of AqPM and OE, BEAS-2B cells were plated at 1.5×10^4 /well in collagen-coated 24-well plates containing 1 ml of BEGM. The cells were allowed to rest for 24 h before being stimulated with AqPM or OE for 16 h in a total volume of 250 μ l/well. Cell viability was determined by trypan blue dye exclusion assay.

2.4. Analysis of IL-6, IL-8 and PGE2

BEAS-2B cells were seeded at 1.5×10^4 /well in collagen-coated 24-well plates containing 1 ml of BEGM and rested for 24 h. The cells were treated with OE (10–100 μ g/ml) in a final volume of 250 μ l/well for 16 h before culture media were collected for analyzing IL-6, IL-8, and PGE2 levels by ELISA. This time point was determined based on previously published reports from DEP studies using bronchial and lung epithelial cells [15,21]. Control groups received equal volume of vehicle. To determine the role of oxidative stress and p38 MAPK in OE-induced IL-6, IL-8, and PGE2 responses BEAS-2B cells were pre-incubated with NAC (1 mM), SFN (5 μ M) or SB203580 (5 μ M) for 2 h before the 16-h OE exposure.

Table 1
Primer sequence for real-time PCR analyses.

Genes	Forward primer	Reverse primer
IL8	TGCCAGCTGTGTGGTAGTG	AAACAAGTTTCAACCAGCAAGA
IL6	GTCCACTGGGCACAGAACTTAT	AAACTGCATAGCCACTTCCAT
IL1B	AAGAGGATCTCCTGTCATCAG	ATAAATAGGGGAAAGCGGTGCTC
HO-1	TCCGATGGGTCCTTACACTC	TAAGGAAGCCAGCCAAGAA
COX2	CAGGAGAAAAGGAAATGTCTGC	AAAAGTGCTTGGCTTCCAGTAG
TSLP	TGAAATCCAGAGCCTAACCTTC	AATTGTGACACTTGTCCAGACA
SOD2	TGTCACCCAGTGGTTTTTGT	GCCCTGCAAATAAACATCTC
ACTIN	AGCACTGTGTTGGCGTACAG	GGACTTCGAGCAAGAGAGG

2.5. Western blot

BEAS-2B cells (1×10^6 /well) in collagen-coated 6-well plates were stimulated with OE (50 $\mu\text{g/ml}$) with or without NAC (5 mM) in 1 ml of BEGM, whereas the control groups receive equal volume of vehicle. HO-1 protein was determined after a 6-h exposure [15,26]. While activation of p38 MAPK was analyzed 2 and 4 h after the addition of OE, COX-2 protein was determined at the 16-h time point [16,21,27]. Cell lysate preparation, protein assay and western blot were performed as previously described [15,27,28]. HO-1, phosphorylated p38, total p38, COX-2, and β -actin proteins were detected using respective primary Abs (1:1000) followed by horseradish peroxidase-conjugated secondary Abs (1:1000). RAW 264.7 cells were obtained from ATCC and cultured in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin (complete DMEM). These cells (10^6 /well) were stimulated with or without DEP suspension (50 $\mu\text{g/ml}$) for 6 h in a 6-well plate containing 1 ml of complete DMEM [26]. Cellular lysates were prepared using previously published method [14].

2.6. Real-time PCR analyses

Total RNA was extracted from BEAS-2B cells following 4-h treatment of 50 $\mu\text{g/ml}$ of OE using an RNeasy Mini kit following the manufacturer's instructions. RNA was eluted in 35 μl of RNase-free water and the concentration was determined using a Nanodrop ($\lambda = 260/280$ nm; ND 1000; Nanodrop Technologies Inc., Wilmington, DE). Total RNA (500 ng) was reverse transcribed to cDNA using High Capacity cDNA Reverse Transcription Kit and relative gene expression was determined by real-time PCR as previously described [29]. Primers used are listed in Table 1.

2.7. Data analysis

Results were expressed as mean \pm standard error of mean (SEM). Differences among groups were evaluated by ANOVA and student *t*-test analysis was used to distinguish between pairs of groups. For the purpose of this discussion, results are considered to be statistically significant for $p < 0.05$.

3. Results

3.1. Pro-oxidative, pro-inflammatory and cytotoxic effects of OE

Consistent with previously published findings chemical analysis revealed that our PM samples collected during charbroiling hamburgers had an OC content of 99% by mass (Fig. 1B) [9]. Trypan blue assay showed that a 16-h exposure to OE (100 $\mu\text{g/ml}$) led to a 30% decrease in cell viability (Fig. 2A). At 50 $\mu\text{g/ml}$, OE induced the expression of antioxidant enzyme HO-1, a highly sensitive marker for PM-induced oxidative stress (Fig. 2B) [23,28,30–34]. Cell lysate from RAW 264.7 cells exposed to a well-characterized collection of DEP was used for comparison [25]. While exposure to DEP suspension (50 $\mu\text{g/ml}$) for 6 h

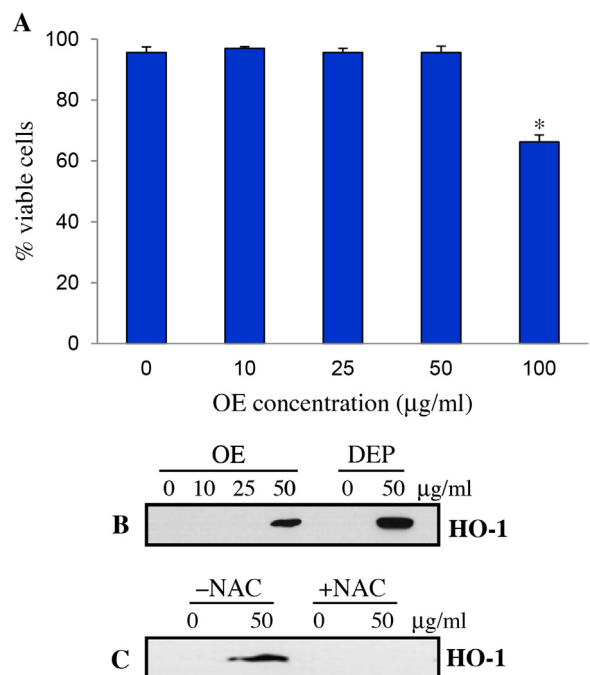


Fig. 2. Induction of cell toxicity and HO-1 expression. (A) The effect of AqPM and OE on cell viability. BEAS-2B cells were exposed to OE at indicated concentrations for 16 h. (B) Induction of HO-1 by OE. Cells were treated with OE at indicated concentrations for 6 h. Cell lysate from RAW 264.7 cells exposed to DEP for 6 h was used for comparison. (C) Effect of NAC on OE-induced HO-1 expression. Cells were pre-incubated with 5 mM of NAC for 2 h before the addition of OE. Values represent means \pm SEM, $n = 3$. * $p < 0.05$ compared to the respective control.

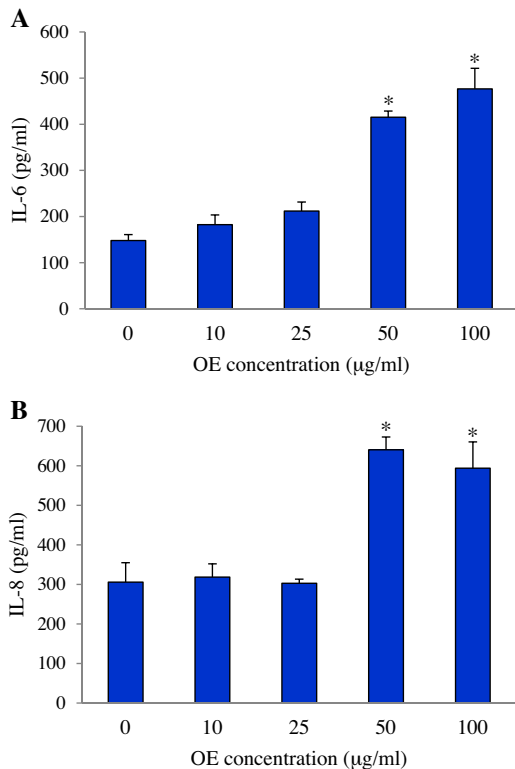


Fig. 3. OE elevated the levels of IL-6 (A) and IL-8 (B) in BEAS-2B cell culture media. Cells were exposed to OE at indicated concentration for 16 h. Values represent means \pm SEM, $n = 3$, * $p < 0.05$ compared to the respective control.

did not have any significant impact on cell viability (95% viable) it had a strong effect on HO-1 expression in RAW 264.7 cells (Fig. 2B). Although not as robust as DEP, OE was quite potent in activating HO-1 and this effect could be completely abolished by the addition of antioxidant NAC (Fig. 2B and C). The inflammatory response of BEAS-2B was determined by measuring IL-6 and IL-8 levels in the cell culture media. ELISA results showed that OE (50 $\mu\text{g/ml}$) could significantly increase the levels of both IL-6 (Fig. 3A) and IL-8 (Fig. 3B). Real-time PCR analysis not only confirmed that HO-1 induction and elevated IL-6 and IL-8 levels were the results of increased gene expression but also identified other genes that were up-regulated in response to OE exposure, including superoxide dismutase 2 (SOD2), IL-1 β , COX2, and thymic stromal lymphopoietin (TSLP) (Table 2).

Table 2
OE-induced gene expression in BEAS-2B (by qPCR).

Cellular response	Genes	Change in expression (fold)
Oxidative stress	HO-1	3.86 \pm 0.62*
	SOD2	1.53 \pm 0.19*
Inflammation	IL-6	2.35 \pm 0.43*
	IL-8	2.29 \pm 0.04*
	IL-1 β	2.74 \pm 0.27*
	COX2	1.68 \pm 0.12*
	TSLP	1.94 \pm 0.30*

Data represent mean fold change \pm SEM, $n = 3$.

* $p \leq 0.05$ when compared to untreated control.

No adverse cellular effects were observed in parallel studies of AqPM (data not shown).

3.2. The role of oxidative stress in OE-induced inflammatory response

To determine whether oxidative stress played a role in OE-induced inflammatory response BEAS-2B cells were treated with OE (50 $\mu\text{g/ml}$) with or without antioxidant NAC or Nrf2 activator SFN. At 1 mM, NAC was sufficient to inhibit OE-induced increase of IL-6 and IL-8 (Fig. 4A and C). A similar inhibitory effect was also observed in BEAS-2B cells that were exposed to OE plus SFN (Fig. 4B and D). In addition, western blot analysis showed that SFN alone could effectively induce HO-1 protein expression in BEAS-2B cells (Fig. 4, inset). Therefore, these results not only confirmed the role of oxidative stress in mediating the inflammatory effect of OE but also suggested that SFN may protect cells against the oxidant activity of OE through the induction of HO-1.

3.3. The role of P38 MAPK in OE-induced IL-6 and IL-8 responses

MAPK including p38 plays an important role in regulating PM-induced inflammatory response in airway epithelial cells [16]. The fact that there is a strong resemblance between BEAS-2B inflammatory responses to OE and vehicular emissions (e.g., DEP) suggests that this signaling pathway may also be involved in the inflammatory effect of OE. To explore this possibility we treated BEAS-2B cells with OE (50 $\mu\text{g/ml}$) for 2 and 4 h, respectively, and analyzed p38 activation by western blot using an antibody against phosphorylated p38. Fig. 5A shows that after 4 h of exposure to OE there was an increase in p38 phosphorylation, while the amount of total p38 remained unchanged, indicating that p38 MAPK could be activated by OE (Fig. 5A). To further confirm the role of p38 MAPK we exposed BEAS-2B cells to OE with or without p38 inhibitor SB203580. Our data demonstrated that SB203580 could significantly lower the levels of IL-6 and IL-8 in the culture media from OE-exposed cells (Fig. 5B and C).

3.4. The impact of OE on COX-2 and PGE2

COX-2 and its downstream product PGE2 are inducible by PM and have been associated with its adverse cellular effects [16,21,35,36]. The 1.68-fold increase in COX-2 gene expression (Table 2) led us to further determine whether this could lead to an increase in PGE2, one of the downstream PG isomers that can be up-regulated in PM-induced inflammation [21,37]. Western blot revealed that after a 16-h exposure there was a profound increase of COX-2 protein in OE-treated cells compared to the control (Fig. 6A). This was accompanied by a 7-fold increase of PGE2 in the culture medium (Fig. 6B). Similar to their effect on OE-induced IL-6 and IL-8, NAC and SB203580 potentially suppressed the stimulatory effect of OE on PGE2. On the contrary, SFN failed to have any impact (Fig. 6B).

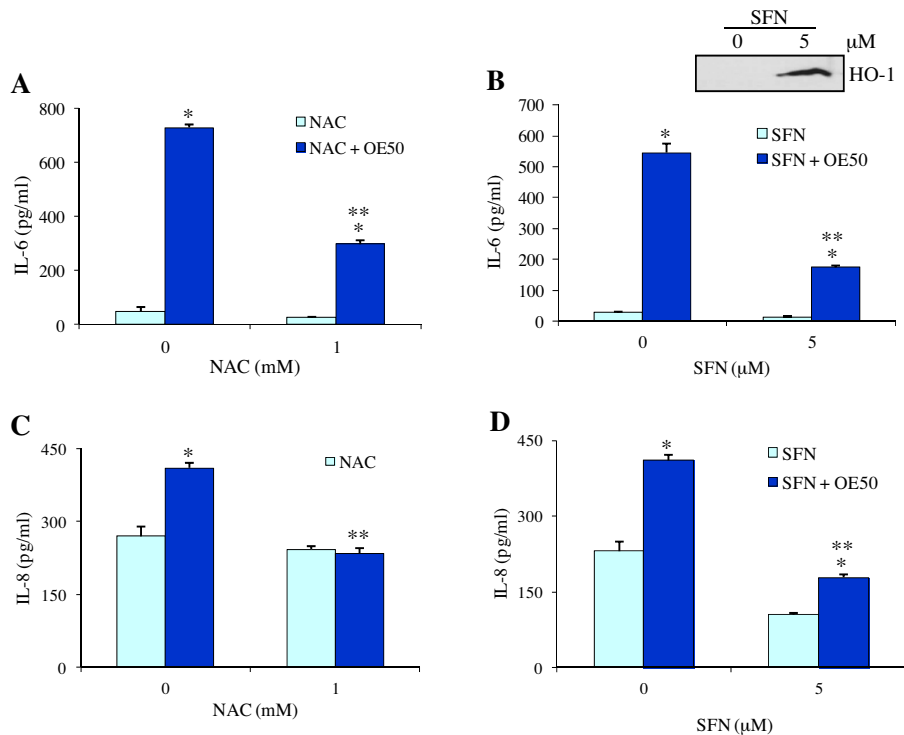


Fig. 4. Inhibition of OE-induced IL-6 and IL-8 production by NAC and SFN. BEAS-2B cells were pre-incubated with or without NAC or SFN for 2 h before exposure to OE for 16 h. (A and B) Effects of NAC and SFN on IL-6. (C and D) Effects of NAC and SFN on IL-8. Inset: Induction of HO-1 expression by SFN after a 6-h exposure. OE50: OE at 50 μg/ml. Values represent means ± SEM, n = 3. *p < 0.05 compared to the respective control; **p < 0.05 compared to OE without NAC.

4. Discussion

In this study, we provide strong evidence demonstrating that organic compounds emitted from commercial-grade charbroiled meat cooking operations could induce an

inflammatory response in human bronchial epithelial cell line BEAS-2B and this effect, as well as its potential mechanisms, was similar to that of traffic-related PM emissions. Our results demonstrate that the organic extract of these emissions could increase inflammatory mediator

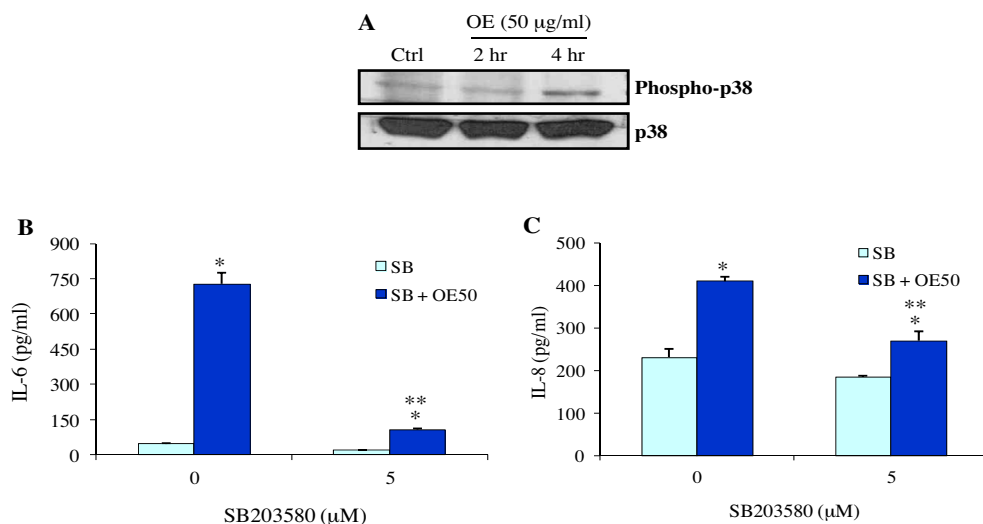


Fig. 5. The role of p38 in mediating the pro-inflammatory effect of OE. (A) Increased p38 phosphorylation in OE-treated BEAS-2B cells. Cells were exposed to OE for 2 and 4 h. Total p38 was used as internal control. (B) Effect of p38 inhibitor on IL-6 production by OE-treated cells. (C). Effect of p38 inhibitor on OE-induced IL-8 production. BEAS-2B cells were pre-incubated with p38 inhibitor SB203580 for 2 h prior to being exposed to OE for 16 h. OE50: OE at 50 μg/ml. Values represent means ± SEM, n = 3, *p < 0.05 compared to the respective control; **p < 0.05 compared to OE alone.

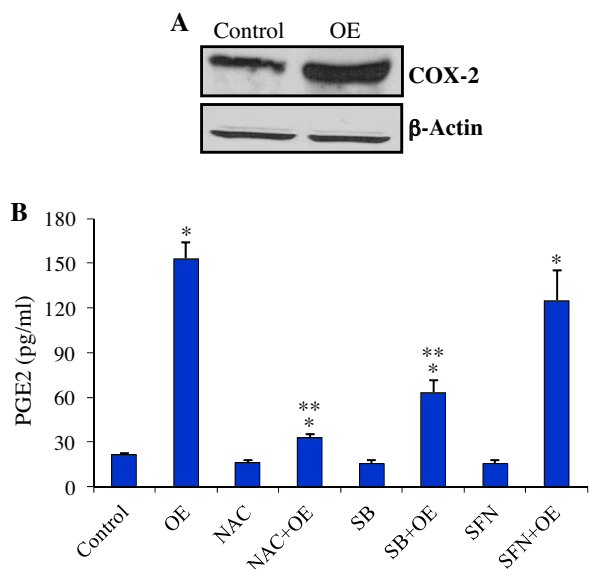


Fig. 6. Up-regulation of COX-2 and PGE2 by OE. (A) Increased COX-2 protein in BEAS-2B cells treated with OE for 16 h. (B) Effects of NAC (1 mM), SFN (5 μ M) and SB203580 (5 μ M) on PGE2 release from BEAS-2B cells after 16-h exposure to 50 μ g/ml of OE. Values represent means \pm SEM, $n = 3$. * $p < 0.05$ compared to the control; ** $p < 0.05$ compared to OE alone.

production (IL-6, IL-8, and PGE2) and these effects of OE were mediated by oxidative stress and p38 MAPK.

PM (e.g. DEP and UFP) with high OC and PAH contents has been shown to induce a stratified cellular oxidative stress response [18,19]. As oxidative stress level increases cellular response progresses from the activation of antioxidant/detoxification defense to inflammation and eventually cell death [18,19]. In our study the presence of cellular oxidative stress in OE-treated BEAS-2B cells was evidenced by the induction of HO-1 and increased SOD2 gene expression (Fig. 2, Table 2). This was further confirmed by the results showing that NAC could completely suppress OE-induced HO-1 activation (Fig. 2). Emissions from charbroiling meat contain numerous organic chemical compounds, many of which are associated with the oxidant effect of DEP [8,10,14,15]. Under our testing conditions meat cooking PM emissions were predominantly OC in nature (Fig. 1B). This is in agreement with previously published report showing that PM emissions from meat cooking operations were primarily composed of OC with only minor concentrations of EC [9]. Thus, the activation of HO-1 and SOD2 likely represented a protective response against the oxidant activity of OC in the emissions.

Redox-active OCs have been identified as the major components that are responsible for the inflammatory effect of traffic-related PM [17,28,35]. A recent study has reported that DEP high in PAH content but low in metals had greater oxidant potential, which was accompanied by a stronger capability to increase IL-6, IL-8, CYP1A1, and HO-1 expression, as well as activating MAPK in BEAS-2B cells [17]. Given its high OC content, the potent effect of OE on IL-6 and IL-8 was consistent with the findings from UFP and DEP studies (Fig. 3) [17,33,35]. The strong inhibitory effect of NAC and SFN confirmed that oxidative

stress played an important role in mediating the inflammatory effect of OE (Fig. 4). A key regulator to protect cells against injuries caused by pro-oxidant air pollutants is transcription factor Nrf2 [38,39]. Nrf2 deficiency can increase the susceptibility to oxidative stress and further strengthen the inflammatory effect of ambient PM that has a high OC content [40]. Pre-treatment of BEAS-2B cells with SFN, a potent Nrf2 activator, can effectively suppress the production of inflammatory mediators (e.g., IL-8, IL-1 β , and MCP-1) induced by air pollutants such as DEP and cigarette smoke [38,39,41,42]. The inhibitory effect of SFN on OE-induced IL-6 and IL-8 demonstrated by our study was consistent with these published findings (Fig. 4B and D). Since SFN alone is highly effective in inducing HO-1 (Fig. 4, inset) it is possible that HO-1 was one of the Nrf2-mediated protective mechanisms that were directly involved in the amelioration of OE-induced inflammatory response in BEAS-2B cells.

p38 MAPK signaling pathway plays an important role in regulating cellular inflammatory responses to PM. Consistent with the findings from studies using PM from other sources, our results showed that OE from meat cooking emissions was able to activate p38, while its inhibitor could suppress the increase of IL-6 and IL-8 from OE-exposed cells (Fig. 5) [16,43–45]. This indicates that p38 MAPK was required for OE-induced inflammatory response, most likely activated by the redox-active OC in the emissions. Other signaling pathways such as JNK, ERK, and NF κ B have also been found to mediate PM-induced inflammation [27,46,47]. Although the present work is limited to investigating p38 MAPK, we are certain that complicated mechanisms involving multiple signaling pathways are responsible for the adverse effects of charbroiling meat emissions because of their complex chemical compositions.

In addition to their effect on promoting inflammatory cytokine/chemokine responses, the organic fraction in charbroiling meat emissions could also elicit a COX-2-mediated inflammatory response involving lipid mediators (Fig. 6). The effects of OE on COX-2 and PGE2 are also in line with the findings from the research in traffic-related PM [16,21,35]. COX-2 is the COX enzyme that is inducible under various conditions including airway inflammation. COX catalyzes the synthesis of PGH₂ from arachidonic acid, which is then used as a main substrate to generate down-stream PG isomers. Compared to other cell types, airway epithelial cells contain a particularly large amount of microsomal PGE synthase that is responsible for PGE2 synthesis. PGE2 plays a role in the early phase of inflammation by acting as a potent local vasodilator [37]. The expression of COX-2 and PGE2 in different cell types including lung epithelial cells has been reported to be p38 MAPK- and NF κ B-dependent and sustained p38 activation is required for COX-2 mRNA stabilization [48,49]. An interaction between p38 MAPK and COX-2 has also been found in human bronchial epithelial cells that were exposed to the DEP with a high PAH content [16]. In cells that are exposed to PM the upregulation of COX-2 and PGE2 may also be mediated through IL-1 β . DEP containing a large amount of PAH and quinones can increase the production of IL-1 β , which has been shown to directly induce COX-2 expression

and PGE2 synthesis in human airway and lung epithelial cells via activation of ERK and p38 MAPK [39,50,51]. Thus, OE-induced 2.74-fold increase of IL-1 β mRNA, together with increased COX-2 and PGE2 levels, suggests that OE may exert its effects on PG synthesis through an IL-1 β -p38 pathway (Table 2, Fig. 6).

Recently, increasing number of studies suggests that TSLP produced by airway epithelial cells may be a key mediator in linking environmental pollutants and allergic airway disease such as asthma [52–55]. It has been demonstrated that TSLP produced by human bronchial epithelial cells upon exposure to air pollutants (e.g., DEP and cigarette smoke) could induce a T-helper 2 immune response through a mechanism involving oxidative stress [52]. Moreover, in a study to assess the association between TSLP and asthma Liu et al. [53] concluded that genetic variants in TSLP may contribute to asthma susceptibility in populations with gene-environment interactions. Therefore, assessing the effect OE on TSLP expression may help us to determine whether exposure to commercial-grade charbroiling meat emissions could potentially exacerbate the respiratory symptoms (e.g., wheezing, nasal allergies, and shortness of breath) reported by commercial kitchen workers [22]. The 1.94-fold increase in TSLP gene expression demonstrated by our PCR analysis is in line with the results from studies using pro-oxidative DEP (Table 2) [52]. This suggests that exposure to OE may contribute to the exacerbation of allergic airway disease (e.g., asthma) through a TSLP-mediated mechanism.

One limitation of this work was that BEAS-2B cells were exposed to AqPM or OE in the culture media. While cellular exposure through air-liquid interface system has the advantage of being more realistic it is not logistically feasible for us at this time. Nonetheless, exposure of BEAS-2B cells to various air pollutants in culture media has been widely and successfully used as an in vitro model for investigating the respiratory effects and their mechanisms of air pollution. Because this was our initial study, we focused on the particle-phase of the emission and limited cellular response pathways in order to determine whether there was a basis for an in-depth research. Based on the findings of this work, we will perform detailed follow-up studies to understand not only the cellular response but also the specific contribution of key chemical components.

5. Conclusion

Emissions from commercial charbroiled meat cooking operations can be an important anthropogenic source of urban air pollution, especially in densely populated cities. Our results provided evidence demonstrating that the organic chemical compounds emitted from these operations could induce oxidative stress and inflammatory responses in human bronchial epithelial cells. These responses and their potential mechanisms were quite similar to those associated with traffic-derived emissions, suggesting that the organic fractions emitted from commercial-grade charbroiling meat operations may pose environmental and occupational health hazards that put residents in the nearby communities and restaurant workers at greater risk. Our data provide strong basis for further

detailed research in the health effects of not only the particles but also the vapors from this source, so that effective control technologies and regulatory guidelines can be developed to reduce environmental and occupational exposures to these emissions.

Conflict of interest

None declared.

Transparency document

The [Transparency document](#) associated with this article can be found in the online version.

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