



Respiratory and cardiovascular effects of ambient particulate matter from dust storm and non-dust storm periods in Kuwait

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Abstract

Epidemiological studies demonstrate a positive association between daily changes in concentrations of ambient airborne particulate matter (PM) and adverse respiratory and cardiovascular health effects. However, physicochemical properties of PM can vary greatly across geographical, atmospheric, and temporal conditions and influence the relative toxicity of airborne PM. The purpose of this study was to investigate the adverse pulmonary and cardiovascular health effects of ambient PM collected from discrete sampling sites in Kuwait during dust storm (DS) and non-dust storm (NDS) conditions. Collected dust samples were characterized for their chemical composition using atomic absorption, GC–MS, and HPLC–MS analyses. Male BALB/cJ mice were exposed to 100 µg of either NDS or dust storm (DS) PM in 50 µl of PBS by oropharyngeal aspiration. Lung function was measured and bronchoalveolar lavage was conducted at 1, 7, and 14 days post-exposure. Ischemia–reperfusion injury was performed 24 h after exposures by obstructing the left main coronary artery approximately 4 mm distal to its origin for 20 min, followed by 2 h. of reperfusion. Exposure to either NDS or DS PM resulted in airway hyperresponsiveness to acetylcholine compared to PBS controls. Total protein and cells in BAL fluid were elevated in both dust groups one day after exposure; however, DS PM induced a greater increase in cell numbers than did NDS PM, particularly in neutrophils, eosinophils, and lymphocytes. Representative lung sections exhibited positive staining for mucus in large airways at 7 days which resolved by 14 days in dust storm-exposed mice but persisted in NDS-exposed animals. Our findings suggest that NDS PM may be more effective in producing an adaptive immune response, while the early inflammation induced by DS PM may better resolve. We also observed a prolonged airway mucus response after exposure to NDS PM, suggesting it may produce more asthma-like features than dust storm PM. PM-induced changes to cardiac ischemia–reperfusion injury were not observed in this study. The lack of cardiovascular response may have been due to the limited exposure and single time point used in this study.

Keywords Airborne dust particulates · Dust storms in Kuwait · Particle size distribution · PM health effects

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Introduction

Over 150 epidemiological studies demonstrate a positive association between daily changes in concentrations of ambient airborne particulate matter (PM) and adverse respiratory and cardiovascular health effects. More than 800,000 excess deaths worldwide each year are attributed to PM. However, physicochemical properties of PM can vary greatly across geographical, atmospheric, and temporal conditions and influence the relative toxicity of PM. Thalib and Al-Ta'iar reported significant association of dust storm events and increases in hospital admissions for asthma and other respiratory conditions in Kuwait (Thalib and Al-Ta'iar 2012). We had the opportunity to investigate the acute toxic effects of ambient PM collected from discrete sampling sites in Kuwait during dust storm and NDS conditions on the cardiopulmonary

system. The purpose of this study was to investigate adverse health effects of Kuwaiti ambient PM collected during dust storms vs NDS periods and their potential contribution to increased risk of respiratory and cardiac illness.

Materials and methods

PM exposure

Male BALB/cJ mice (Jackson Labs, Bar Harbor, ME) at 7 weeks of age were anesthetized with isoflurane and subject to oropharyngeal aspiration of 100 µg of either NDS DS PM in 50 µL of PBS or 50µL of PBS alone $n=18$ mice/exposure group. Mice in each exposure group were later euthanized at three separate time points ($n=6$ mice/exposure group/time point).

Lung function

Lung function measurements were made 1, 7, or 14 days post-exposure using the flexiVent system (SCIREQ, Montreal, QC, Canada). Animals ($n=6$ /exposure group/time point) were anesthetized (~400 mg/kg tribromoethanol, i.p.), tracheostomized, and ventilated with room air at 10 ml/kg and 150 breaths per minute with positive end expiratory pressure of 3 cm H₂O. Respiratory mechanics were measured using a script to ensure consistent timing of perturbations relative to total lung capacity maneuvers to standardize volume history. Respiratory system resistance (R) was measured at baseline and after increasing concentrations of aerosolized acetylcholine.

Bronchoalveolar lavage

Immediately after lung function measurements, mice were exsanguinated, the left bronchus was clamped, and the right lung was lavaged with four successive aliquots of 26.25 ml/kg cold Hanks' balanced salt solution (HBSS). Recovered lavage fluid (BALF) was centrifuged at 500×g for 10 min at 4 °C. Supernatant from the first BALF aliquot was used for measurement of total protein. All cells from the same individual were pooled, and total cell counts were made with a hemocytometer. Slides were prepared by cytocentrifugation of 20,000 cells at 600 rpm for 5 min (Cytospin III, Shandon) and stained (Richard-Allan, Kalamazoo, MI). BALF cell differential counts were performed on 300 cells per slide using standard morphological criteria.

Quantitation of protein in BALF

Total protein concentration in BALF (an index of lung permeability and lung injury) was measured using a Bradford

protein assay (BioRad, Hercules, CA) according to manufacturer's recommendations and read at 595 nm on a spectrophotometer (Beckman Coulter, Brea, CA). Concentration was calculated from a standard curve.

Measurement of lung collagen

Lavaged right lung lobes were homogenized in RIPA lysis buffer plus protease inhibitor cocktail and PMSF (Sigma-Aldrich, Milwaukee, WI). Lung homogenates were centrifuged at 10,000×g for 10 min at 4 °C. Soluble collagen was measured by adding 500 µl Sircol dye reagent (Biorcolor Ltd., Carrickfergus, UK) to 50 µl of lung homogenate supernatant. Samples and standards were agitated for 30 min and then centrifuged at 10,000×g for 10 min to pellet collagen-bound dye. Supernatants were discarded, and pellets were dissolved in 1 ml 0.5 M NaOH. Absorbance was read at 540 nm, and sample concentrations were calculated from a standard curve.

Lung histology

Left lungs were inflated with 10% neutral buffered formalin (Azer Scientific, Morgantown, PA) for 24–72 h before cutting into three sections and undergoing standard processing. Five-µm cross sections were placed in xylene to remove paraffin, hydrated through an ethanol gradient, and stained with Masson's trichrome to allow visualization of collagen and Alcian Blue–periodic acid–Schiff (AB-PAS) for mucoid substances.

Cardiovascular ischemia–reperfusion (I/R) injury

An established protocol was used to measure I/R injury (Cozzi et al. 2006).

Statistical analysis

All data are presented as the mean ± SEM for each group. Two-way analysis of variance (ANOVA) was used to evaluate exposure and temporal effects. Bonferroni post hoc testing was used to determine statistical differences between groups, and $p < 0.05$ was accepted as statistically significant. SigmaStat 3.0 software (SPSS Science Inc., Chicago, IL) was used for all statistical analyses.

Results and discussion

Lung function

Airway resistance increased with increasing concentrations of aerosolized acetylcholine. Exposure to 100 µg of

either NDS or DS PM resulted in hyperresponsiveness to the higher concentrations of acetylcholine compared to PBS controls at day 1 (Fig. 1). At days 7 and 14 post-exposure, there were no significant differences among exposure groups (data not shown).

BAL protein and cells

Protein concentrations were significantly elevated in mice exposed to both NDS and DS PM compared to PBS controls one day after exposure, but were not different from PBS controls thereafter (Fig. 2). Similarly, the total number of cells recovered in BAL fluid increased significantly one day after exposure to NDS and DS PM compared to PBS controls, but was not different from controls at later time points (Table 1). Specific cell types differed widely depending on type of PM exposure and time point. Macrophages accounted for approximately 90% of BAL cells in PBS-exposed mice across all time points. Exposure to NDS PM elicited an increase in macrophage numbers at 14 days which was not seen in DS PM-exposed mice. There were no significant differences in macrophage numbers in any exposure group at 1 and 7 days post-exposure. The pattern of epithelial cells mirrored that of BALF protein with significant increases in both PM-exposed groups at one day, but no differences from controls at subsequent time points. Lymphocytes, neutrophils, and eosinophils were elevated in both NDS- and DS-exposed mice at one day, but to a significantly greater extent in DS-exposed mice. Lymphocytes and eosinophils were also elevated at 14 days post-PM exposure, but to a greater extent in NDS-exposed mice.

Histological evaluation of lung sections from mice exposed to Kuwaiti PM showed mild increases in airway mucus content in large airways at 7 days post-exposure

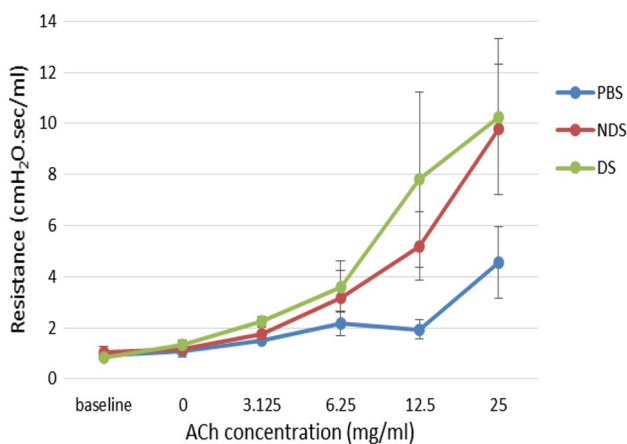


Fig. 1 Mice exposed to Kuwaiti NDS and DS PM are hyperresponsive to increasing concentrations of aerosolized acetylcholine compared to PBS controls one day after PM exposure. $N=6$ mice/exposure group

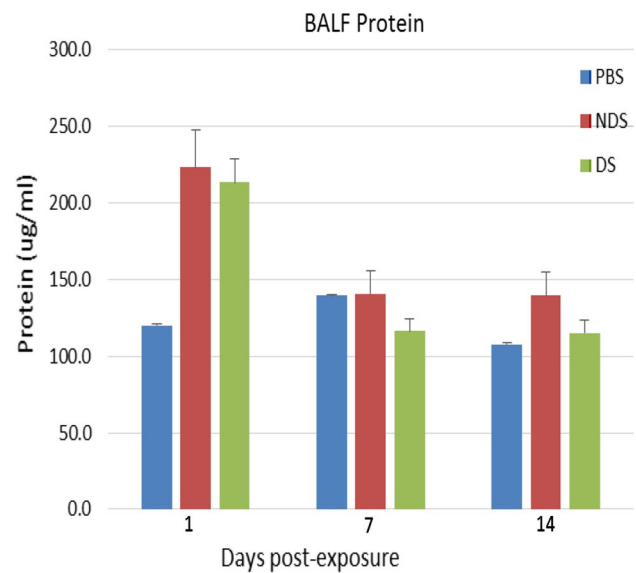


Fig. 2 Total protein content in bronchoalveolar lavage fluid. At one day post-exposure, both NDS and DS PM induced elevated protein levels indicative of increased lung permeability and injury. $*p < 0.05$ vs PBS within time point

(Fig. 3). Mucus production subsided by 14 days post-exposure in DS-exposed mice but was still present in NDS-exposed mice. Lung collagen content did not differ significantly in any exposure group or time point (data not shown), nor were increases seen in lung collagen histologically.

Conclusion

We found that aspiration of equal masses of Kuwaiti PM collected during NDS and dust storm periods elicited largely similar pulmonary responses, but with some interesting temporal differences. Both types of PM elicited increased airway responsiveness to acetylcholine provocation as well as increases in lung permeability one day after exposure. Additionally, both types of PM induced cellular inflammation in the lungs with increases in lymphocytes, neutrophils, and eosinophils; however, this inflammatory response was greater in DS-exposed compared to NDS-exposed mice one day after exposure. Interestingly, increased numbers of lymphocytes and eosinophils were also seen at 14 days post-exposure, but to a greater extent in NDS-exposed mice at this time point. Furthermore, macrophage numbers were also elevated in NDS-exposed mice at 14 days. These findings suggest that NDS PM may be more effective in producing an adaptive immune response in naïve mice, while the early inflammation induced by DS PM may better resolve.

We also observed a prolonged airway mucus response after exposure to NDS PM, suggesting it may produce more asthma-like features in naïve mice than DS PM.

Table 1 Cells recovered from bronchoalveolar lavage fluid after PM exposure

Exp	Day	Total cells	Macrophages	Epithelial cells	Neutrophils	Eosinophils	Lymphocytes
PBS	1	75,417 ± 17,241	49,148 ± 18,861	4424 ± 730	9514 ± 5696	2607 ± 2225	629 ± 261
	7	95,000 ± 15,838	87,482 ± 15,008	5583 ± 1069	1015 ± 793	439 ± 173	481 ± 143
	14	88,750 ± 3146	83,193 ± 3040	4542 ± 286	50 ± 50	260 ± 101	706 ± 177
NDS	1	214,375 ± 33,593	58,710 ± 10,695	8638 ± 2373	112,500 ± 63,557	6288 ± 2156	2306 ± 773
	7	124,583 ± 18,612	113,233 ± 17,320	5135 ± 1424	4235 ± 1086	1236 ± 165	744 ± 183
	14	145,417 ± 17,386	122,653 ± 17,431	6304 ± 562	8165 ± 1318	3561 ± 977	4733 ± 1170
DS	1	280,833 ± 42,410	77,007 ± 15,704	9161 ± 1553	178,379 ± 23,465	9171 ± 2996	7115 ± 1670
	7	106,667 ± 10,986	99,043 ± 10,115	4776 ± 1172	1135 ± 491	933 ± 384	779 ± 175
	14	98,750 ± 9995	85,550 ± 9322	4721 ± 588	3126 ± 493	1706 ± 362	3647 ± 446

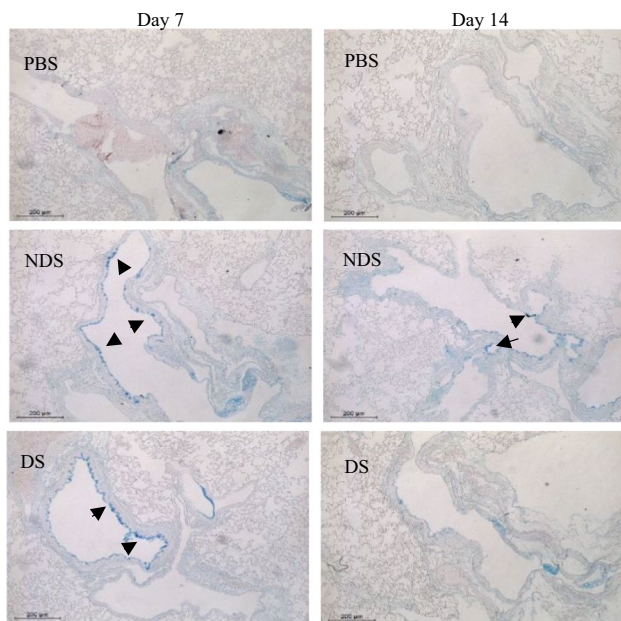


Fig. 3 Representative lung sections from mice exposed to PBS, NDS PM, or DS PM. Airways from mice exposed to PBS (top row) did not stain for mucus, while NDS PM-exposed mice exhibited positive staining for mucus in large airways (bronchi) at 7 and 14 days post-exposure. Airways from DS PM-exposed mice stained positive for mucus at 7 days, but this effect had mostly resolved by 14 days. Arrows indicate bronchial epithelium that stained positive for mucus. Alcian Blue–periodic acid–Schiff staining of 5- μ m sections. $\times 100$ original magnification

An epidemiological study found that hospitalizations for asthma were associated with dust storm events in Kuwait (Thalib and Al-Taiar 2012). Our data showing greater inflammation in response to DS PM support the epidemiological evidence; however, NDS PM from Kuwait may contribute to the development of asthma in this region, while DS PM may exacerbate existing respiratory conditions such as asthma. Investigation of the differential effects of these different types of PM in mouse model of allergic

asthma would better answer such questions. In addition, an analysis of the physical and chemical composition of the PM may assist in explaining the observed differences. The increases in epithelial cell numbers recovered in lavage fluid one day after PM exposure which are in line with the increases in protein content suggest that aspiration of these dusts may induce injury to the epithelial and endothelial barriers in the lungs. Although PM-induced changes to cardiac ischemia–reperfusion injury were not observed in this study, further exploration of cardiovascular effects may be warranted.

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