Lung pathobiology after exposure to source-oriented particulate matter (PM) from the Central Valley of California in BALB/c mice

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Introduction
There is growing evidence that exposure to particulate matter (PM) can lead to adverse health effects in both humans and animals. These effects are primarily seen as lung inflammation and injury associated with increased risk of exacerbating the incidence and severity of asthma, chronic obstructive pulmonary disease (COPD), and respiratory infection. The Central Valley of California was chosen as the site to conduct this study because it consistently has high levels of ambient PM and asthma rates. Previous studies used bronchoalveolar lavage (BAL) to measure inflammation caused by PM of different sizes, types, and season. This study uses histopathology scoring and gene expression analysis to show specific cellular, inflammatory and structural changes in the lungs of BALB/c mice following acute exposure to Central Valley PM. The extent and severity of alveolitis, bronchiolitis, perivasculitis, and pleuritis were histopathologically scored. The gene expression of cytokines, chemokines, and oxidative stress enzymes was analyzed.

Objective/Hypothesis

Objective: To find an association between the degree of lung inflammation indicated from BAL, histopathology and gene expression.

Hypothesis: Particulate matter (PM) of different sizes, chemical composition, and season will produce different degrees of lung inflammation, injury, and cytokine/chemokine activation. The patterns of biological response will be similar between BAL, histopathology, and gene expression.

Materials and Methods

Animals: Male BALB/c mice (9-10 wk old) were obtained from Charles River Laboratories, Inc. All animal protocols were approved by IACUC of the University of California, Davis. Mice were exposed via oropharyngeal aspiration to the equivalent mass dose [50 μg] of two size fractions: ultrafine (UF: Dp <0.17 μm) and submicron fine (SMF: 0.17 μm -1 μm) during summer and winter seasons. Lungs were collected 24 hours post-exposure.

Histopathology Scoring: Left lung lobes were fixed/infiltrated with 4% paraformaldehyde, embedded in paraffin, sectioned into 5μm slices and staining with H&E. Pathological assessment was performed on cranial, hilar, subhilar, and caudal regions using a scoring system based on the extent and severity of alveolitis, bronchiolitis, perivasculitis, and pleuritis. Extent and severity were both assessed using a scale ranging from 0 (no inflammation), 1 (mild inflammation), 2 (marked inflammation), 3 (extensive inflammation), to 4 (severe inflammation). Total score = extent x severity. The cumulative score was the sum of the average value of each parameter.

qPCR was performed with Fast SYBR Green PCR Mastermix (Applied Biosystems) using primers for the following genes: 1) cytokines (IL-1β, IL-17a, TNF-α), 2) chemokines (CCL1, CCL3, CCL4, CXCL2, CXCL5), and 3) oxidative stress enzymes (DUOX1, GPX1, and HMOX1).

Statistical Method: Treatment group comparisons consisted of 1) vehicles (gas and diesel) UF in summer, 2) vehicles (gas and diesel) SMF in summer, 3) source mixture UF in summer, and 4) vehicles (gas and diesel) UF in winter that were assessed by one way ANOVA followed by post hoc Tukey HSD. Data are expressed as mean ± standard error of the mean (SEM). A value of p<0.05 was considered significant.

Results

Cumulative Histopathology Score vs. Bronchoalveolar Lavage

Figure 1. Cumulative score from histopathology (left) and BAL (right).

Histopathology Scoring Profile

A. Seasonal effect

B. Particle Size Effect

C. Chemical Composition effect

Gene Expression Profile

A. Cytokines

B. Chemokines

C. Oxidative Stress Enzymes

Figure 3. Gene expression profiles of A) cytokines, B) chemokines, and C) oxidative stress enzyme from the right lung of mice. Results are presented as mean ± SEM. *, p<0.05; **, p<0.01; ***, p<0.001.

Histopathology images

Figure 4. Histopathology images of alveolitis, bronchiolitis, perivasculitis, and pleuritis. Arrows indicate inflammatory cells that were used to determine the score of inflammation.

Conclusion and Future Directions

- Cumulative histopathology scoring (Figure 1) demonstrates a similar pattern found in the BAL in terms of cell inflammation for PM size, chemical composition, and seasons.
- Vehicles (gas and diesel) ultrafine in summer (Figure 2) caused the most severe alveolitis, bronchiolitis, and perivasculitis with significant difference in alveolitis and perivasculitis for PM seasonal effect, particle size effect, and chemical composition effect. This result is similar to that observed by BAL analysis of inflammation. Perivasculitis scored the highest perhaps due to inflammatory cells coming from the vascular circulation.
- Vehicles (gas and diesel) ultrafine in summer (Figure 3) had significant difference in cytokines (IL-1β and TNF-α), chemokines (CCL3, CCL4, CXCL2, and CXCL5), and oxidative stress enzymes (DUOX1, GPX1, and HMOX1). These cytokines and chemokines influence the influx of neutrophils (CCL3 and CXCL5) and macrophages (CCL3, CCL4, CXCL2, IL-1β, TNF-α) in lung tissue. The gene expression was supported by BAL and histopathology results because vehicles (gas and diesel) ultrafine in summer produced a high number of inflammatory cells recovered in BAL and the highest score of inflammation in histopathology scoring.
- The degree of lung inflammation, injury, and cytokine/chemokine activation differed based on the size, season, and chemical composition of PM. Also, BAL, histopathology scoring, and gene expression showed similar patterns in that vehicles (gas and diesel) ultrafine in summer was the highest and significantly different in the three experiments. Thus, vehicles (gas and diesel) ultrafine in summer has the potential to cause severe lung inflammation and should be regulated more rigorously.
- Future experiments would be to assess cytokine, chemokine, and oxidative stress enzyme protein levels with ELISA and immunohistochemistry. A larger sample of genes could also be measured with PCR to assess lung inflammation.

References

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