Serum Neurofilament Light Polypeptide is a Biomarker for Inflammation in Cerebrospinal Fluid Caused by Fine Particulate Matter

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ABSTRACT

Epidemiological and toxicological evidence indicates that fine particulate matter (PM_{2.5}, particulate matter < 2.5 μm in aerodynamic diameter) causes cardiopulmonary toxicity; however, its neurotoxic effects remain unclear. The objective of this study was to investigate the role of the neurofilament light (NEFL) serum polypeptide in neurotoxicity. 6-month-old male Sprague Dawley (SD) rats were exposed to traffic-related PM_{1} (< 1 μm in aerodynamic diameter; 16.3 μg m^{-3}) and gaseous pollutants (via high-efficiency particulate air, HEPA) for 3 and 6 months through a whole-body exposure system. According to our observations, the levels of interleukin (IL)-4, IL-10, and tumor necrosis factor (TNF)-α in the serum of the rats significantly increased (p < 0.05) after 3 months of exposure to PM_{1}, whereas that of NEFL polypeptide significantly increased (p < 0.05) after 3 and 6 months of exposure. Additionally, increases in the IL-2, IL-6, IL-10, IL-17α, TNF-α, and interferon (IFN)-γ levels after 3 and/or 6 months of exposure to this pollutant (p < 0.05) were observed in the cerebrospinal fluid (CSF). In terms of their respective levels, the IL-6 correlated well with the CSF IL-2 and IL-10; the TNF-α correlated well with the CSF IL-6, IL-17α, TNF-α, and IFN-γ; and the NEFL polypeptide correlated well with the CSF IL-2, IL-4, IL-6, IL-10, IL-17α, TNF-α, and IFN-γ. In summary, systemic neuroinflammatory and immune responses in rats occurred after chronic exposure to PM_{1}. Hence, NEFL polypeptide in serum may be a suitable biomarker for neurotoxicity caused by chronic exposure to this pollutant.

Keywords: Air pollution; Central nervous system; Neurotoxicity; Oxidative stress; Particulate matter.

INTRODUCTION

Neurodegenerative diseases are emergent chronic diseases in aging populations nowadays. According to the National Institute of Neurological Disorders and Stroke, there are more than 600 neurodegenerative disease, which affect approximately 50 million Americans each year (Brown et al., 2005). In Taiwan, the average age-standardized prevalence of neurodegenerative diseases, such as Parkinson’s disease (PD), per 100,000 population were 84.8 in 2004 and 147.7
in 2011, representing a 7.9% annual increase (Liu et al., 2016). Notably, accumulating epidemiological evidence indicates that pulmonary exposure to particulate air pollution is associated with increased risks of neurodegenerative diseases (Power et al., 2011). For example, exposure to high levels of coarse particulate matter (PM\(_{2.5-10}\)) and fine particulate matter (PM\(_{2.5}\)) is associated with increased risks of cognitive decline (Weuve et al., 2012). Increased risks of neurodegenerative diseases such as PD were associated with chronic exposure to air pollution (Kioumourtzoglou et al., 2009). Therefore, an Aethalometer (AE33; Magee Scientific, Berkeley, CA, USA) was used to measure black carbon (BC) mass concentration. Additionally, the numbers of cars were manually calculated to obtain passenger car units (PCU) during the study period.

METHODS

Experimental Design

Male 6-month-old Sprague Dawley (SD) rats obtained from the National Laboratory Animal Center (Taipei, Taiwan) were used throughout the study. Rats were randomly selected for three groups: high-efficiency particulate air (HEPA; exposed to gaseous pollution only; \(n = 12\); New Taipei City, Taiwan), and PM\(_1\) exposure to traffic-dominated air pollution (exposed to particulate and gaseous pollution; \(n = 12\); New Taipei City, Taiwan). The HEPA group (gaseous pollution only) served as control, whereas the PM\(_1\) (particulate and gaseous pollution) served as exposure. The exposure site was a traffic-dominant area, which was near a highway (760 m) and an expressway (76 m). The distance to the closest major road was approximately 62 m. Rats were housed at a constant temperature of 22 ± 2°C and a relative humidity (RH) of 55 ± 10% with a 12:12-h light:dark cycle throughout the study. After 3 and 6 months of exposure, rats were necropsied, and serum and CSF were collected from the nearby Taiwan Environmental Protection Administration Yonghe air quality monitoring station, and the concentrations and physical properties of PM were characterized by the collocated instruments. In the system, a tapered element oscillating microbalance (TEOM; Model 1400a; Thermo Scientific, USA) was used to determine PM\(_1\) mass concentrations. A Scanning Mobility Particle Sizer (SMPS; Model 3936; TSI Inc., USA) and an Aerodynamic Particle Sizer (APS; Model 3321; TSI Inc., USA) were used to examine the submicron particle size distribution (PSD) and supermicron PSD, respectively. A Nanoparticle Surface Area Monitor (NSAM; Model 3550; TSI Inc., USA) was used to monitor lung deposition surface area (LDSA) concentrations. It was reported that measurements of black carbon (BC) by continuous monitors, such as an Aethalometer, showed good correlations with concentrations of traffic exhaust particles or with traffic counts (Wu et al., 2007; deCastro et al., 2008; Patel et al., 2009). Therefore, an Aethalometer (AE33; Magee Scientific, Berkeley, CA, USA) was used to measure the BC mass concentration. Additionally, the numbers of cars were manually calculated to obtain passenger car units (PCU) during the study period.
The average daily traffic flows in the major commuting routes are all over 50,000 PCU day\(^{-1}\). The Shuang Ho area (New Taipei City, Taiwan), the neighborhood of the two major crossing/commuting avenues, was set as the monitoring site which was located in a trailer station at about 6 m above the ground. The sampled PM\(_{1}\) was considered exposure to gaseous pollutants only, and the other system without filters was the experimental group that was exposed to PM\(_{1}\) as well as gaseous pollutants.

As shown in Fig. 1, the penetration of particles in the IVC without a filter was 75% over the entire particle size range, and the average particle penetration was 80% for particles of \(< 550 \text{ nm in size. In the particle size range ranging 550–1000 nm, the average particle penetration was 34% and gradually decreased to about 20%. In other words, the major particles to which the mice were exposed were PM\(_{1}\). For the IVC with filters, the average penetration of particles was 4% over the entire particle size range. Particularly for particles of \(> 600 \text{ nm}, the overall removal efficiency of particles could be as high as 99%. The typical exposure PSDs measured in the exposure chamber/cabinet of the IVC are shown as a number and on a mass basis (Figs. 2(a) and 2(b)). The number PSD demonstrates the typical single Aitken-mode traffic characteristics with a major peak size of around 49 nm and a total number concentration of around \(10^{4–10^{5}} \text{ particles cm}^{-3}\). When an identical PSD was shown in the volume concentration, the typical tri-mode for PM\(_{1}\) was observed, and the peak size of the volume PSD was located at 400 nm. Thus, the total mass concentration of PM\(_{1}\) should largely be attributed to these accumulation modes, and the main chemical components could be sulfate, nitrate, ammonium, organic carbon, elemental carbon, and heavy metals (Tsai et al., 2012; Liang et al., 2013; Lin et al., 2015).

Table 1 shows the air pollution to which rats were exposed during the study period. The PM\(_{1}\), PNC, BC, and LDSA were 16.3 ± 8.2 µg m\(^{-3}\), 11,257 ± 4388 particles cm\(^{-3}\), 1800 ± 784 ng m\(^{-3}\), and 55.1 ± 21.7 µm\(^{2}\) cm\(^{-3}\), respectively, which were previously reported (Shih et al., 2018). In the present study, the rats were exposed to relatively lower levels of PM\(_{1}\) than the WHO PM\(_{2.5}\) guideline (25 µg m\(^{-3}\) for a 24-h average) (WHO, 2006). The PNC and BC presented higher levels during the experiment period than previous measurements (Chuang et al., 2017). This observation suggested that traffic-related particulate pollutants were the main source of exposure for rats. As to pollutants obtained from the air quality station, the PM\(_{10}\), PM\(_{2.5}\), NO\(_2\), SO\(_2\), and O\(_3\) levels were all below WHO guidelines (WHO, 2006). Therefore, the exposure conditions provided a good environment to study the effects of long-term exposure to lower levels of air pollution on neurotoxicity in vivo. 

**Results and Discussion**

**Chronic Exposure to PM\(_{1}\)**

Exposure to Air pollution has been linked to increased risks of neurodegenerative diseases (Malek et al., 2015; Lee et al., 2019). To study NEFL polypeptide expression in serum after chronic exposure to PM\(_{1}\), an \textit{in vivo} whole-body exposure system was established for chronic exposure to PM\(_{1}\). Traffic is one of the most prominent sources contributing to airborne PM in urban environments, and a trailer-type monitoring site was established which was located in a neighborhood of the two major crossing/commuting avenues in the Shuang Ho area (New Taipei City, Taiwan), a typical urban environment in Taiwan. The average daily traffic flows in these major commuting routes are all over 50,000 PCU day\(^{-1}\). The average daily traffic volume was 444 cars h\(^{-1}\) and 4731 motorcycles h\(^{-1}\) for evening rush hour (17:00–20:00) and 462 cars h\(^{-1}\) and 2183 motorcycles h\(^{-1}\) for morning rush hour (06:00–08:00) (Supplementary Material Fig. S1).

Sampling inlets were located at the top of the roof of the trailer station at about 6 m above the ground. The sampled traffic-related air pollution was delivered to two modified individual ventilated cage (IVC) systems. Rats were whole-body exposed to PM\(_{1}\) using a whole-body exposure system for 6 months as reported previously (Shih et al., 2018). One system with filters was considered exposure to gaseous pollutants only, and the other system without filters was the experimental group that was exposed to PM\(_{1}\) as well as gaseous pollutants.
Fig. 1. Illustration of the airflow of the whole-body exposure system for the *in vivo* experiment. Average penetration rates of the filtered group and unfiltered group at different particle size ranges.

Fig. 2. (a) Typical exposure number particle size distribution (PSD); (b) typical exposure volume PSD.

Pulmonary disease (COPD) (Ho *et al.*, 2019). Therefore, PM$_{10}$ exposure may alter systemic immune responses after subchronic exposure in rats.

We noted that serum IL-10 had increased after 3 months of PM$_{10}$ exposure compared to the controls ($p < 0.05$), whereas there was no significant difference in IL-6 between the controls and the PM$_{10}$-exposed group (Fig. 3). The results indicated that an anti-inflammatory reaction was activated to mitigate the PM$_{10}$-induced systemic inflammation in rats. Inflammation was resolved by the self-repair/defense response; thus, persistent low-grade inflammation in serum after chronic exposure to PM$_{10}$ was observed in our study. Notably, we investigated levels of the NEFL polypeptide in serum after PM$_{10}$ exposure (Fig. 3). The NEFL polypeptide is unique to neuronal cells, and it is shed to the CSF and is detectable at low concentrations in peripheral blood (Disanto *et al.*, 2017). The presence of increasing levels of the NEFL polypeptide in the CSF is considered to be an indicator of axonal damage in the CNS (Norgren *et al.*, 2003). Recently, the NEFL polypeptide was detected in serum in the presence of a neurodegenerative disease (Norgren *et al.*, 2003). Levels of the NEFL polypeptide showed good correlations between CSF and serum samples, both of which are able to represent the disease progress of neurodegenerative diseases (Disanto *et al.*, 2017). In the present study, the NEFL polypeptide had significantly increased ($p < 0.05$) after 3 and 6 months of exposure to PM$_{10}$ compared to the controls. Increased levels of the NEFL polypeptide suggested that chronic exposure to...
PM\(_1\) led to an increase in its levels in the serum and CSF, which could be associated with the development of neurodegenerative diseases by PM\(_1\). However, more studies are required to investigate the role of the NEFL polypeptide in response to air pollution exposure.

**Biomarkers Determined in CSF**

CSF is commonly used to determine levels of neuroinflammation and oxidative stress and to represent the healthy or diseased statuses of the brain (Guest et al., 2014). In the present study, we observed that IL-17\(\alpha\) and TNF-\(\alpha\) had increased with 3 months of PM\(_1\) exposure, and IL-6 had increased with 6 months of PM\(_1\) exposure (\(p < 0.05\); Fig. 4). Both 3 and 6 months of exposure to PM\(_1\) caused increases in IL-2, IL-10, and IFN-\(\gamma\) levels in the CSF compared to the controls (\(p < 0.05\); Fig. 4). The results indicated that neuroinflammation occurred due to chronic exposure to PM\(_1\) as observed in the CSF. Similarly, alterations in CSF inflammatory markers due to air pollution were investigated in children and adults in Mexico City (Calderon-Garciduenas et al., 2016). They observed that PM\(_{2.5}\) exposure increased the risk of AD and PD. A previous study further observed that neuroinflammation occurred in children of Mexico City after exposure to severe air pollution, leading to IL-6 and IL-2 increases in CSF (Calderon-Garciduenas et al., 2013). Smaller combustion-derived particles, especially ultrafine PM, induced more-significant impacts in the brains of children (Gonzalez-Maciel et al., 2017). Therefore, alterations in inflammatory responses of CSF may be able to represent impairment of the brain caused by PM\(_1\). However, the contributions of physicochemistry of PM to CSF inflammation require further investigation.

**Correlations of Biomarkers between Serum and CSF**

CSF biomarkers were correlated with levels of inflammation in serum. In the present study, associations of biomarkers between serum and CSF were examined as shown in Table 2. We observed that serum TNF-\(\alpha\) was correlated with serum NEFL polypeptide (\(r = 0.477, p < 0.05\)). Serum IL-6 was correlated with CSF IL-2 (\(r = 0.520, p < 0.01\)) and IL-10 (\(r = 0.478, p < 0.05\)). Serum TNF-\(\alpha\) was correlated with CSF IL-6 (\(r = 0.522, p < 0.01\)), IL-17\(\alpha\) (\(r = 0.519, p < 0.01\)), TNF-\(\alpha\) (\(r = 0.519, p < 0.01\)), and IFN-\(\gamma\) (\(r = 0.407, p < 0.05\)).

<table>
<thead>
<tr>
<th>Biometric and pollutants (unit)</th>
<th>Mean ± SD</th>
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<tbody>
<tr>
<td><strong>Particulate matter</strong></td>
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<tr>
<td>PM(_1) ((\mu g) m(^{-3}))</td>
<td>16.3 ± 8.2</td>
</tr>
<tr>
<td>PNC (particles cm(^{-3}))</td>
<td>11.257 ± 4388</td>
</tr>
<tr>
<td>BC (ng m(^{-3}))</td>
<td>1800 ± 784</td>
</tr>
<tr>
<td>LDSA ((\mu m^2) cm(^{-3}))</td>
<td>55.1 ± 21.7</td>
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<tr>
<td><strong>Ambient pollutants</strong></td>
<td></td>
</tr>
<tr>
<td>PM(_{10}) ((\mu g) m(^{-3}))</td>
<td>36.6 ± 15.2</td>
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<tr>
<td>PM(_{2.5}) ((\mu g) m(^{-3}))</td>
<td>19.7 ± 9.8</td>
</tr>
<tr>
<td>NO(_x) (ppb)</td>
<td>32.9 ± 16.4</td>
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<tr>
<td>SO(_x) (ppb)</td>
<td>2.5 ± 1.0</td>
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<td>O(_3) (ppb)</td>
<td>29.7 ± 11.0</td>
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1. PM data referenced from Shih et al. (2018).
2. Gaseous pollution was measured by the Environmental Protection Administration Yonghe air quality monitoring stations.

PM\(_1\): particulate matter of < 1 \(\mu\)m in aerodynamic diameter; PNC: particle number concentration; BC: black carbon; LDSA: lung deposition surface area; PM\(_{10}\): particulate matter of < 10 \(\mu\)m in aerodynamic diameter; PM\(_{2.5}\): particulate matter of < 2.5 \(\mu\)m in aerodynamic diameter; NO\(_x\): nitrogen dioxide; SO\(_x\): sulfur dioxide; O\(_3\): ozone.
Fig. 3. Interleukin (IL)-4, IL-6, IL-10, tumor necrosis factor (TNF)-α, and the neurofilament light (NEFL) polypeptide were determined in the serum of rats in the control, HEPA (high-efficiency particulate air), and PM1 (particulate matter with an aerodynamic diameter of < 1 µm) groups (n = 6). * p < 0.05.
Interleukin (IL)-2, IL-4, IL-6, IL-10, IL-17a, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ were determined in the cerebral spinal fluid (CSF) of rats in the control, HEPA (high-efficiency particulate air), and PM₁ (particulate matter with an aerodynamic diameter of <1 µm) groups (n = 6). * p < 0.05.
Table 2. Correlations of biomarkers between the serum and cerebral spinal fluid (CSF).

<table>
<thead>
<tr>
<th>Serum</th>
<th>IL-4</th>
<th>IL-6</th>
<th>IL-10</th>
<th>TNF-α</th>
<th>NEFL</th>
<th>IL-2</th>
<th>IL-4</th>
<th>IL-6</th>
<th>IL-10</th>
<th>IL-17α</th>
<th>TNF-α</th>
<th>IFN-γ</th>
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<td>CSF</td>
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- **Correlation is significant at the 0.01 level (2-tailed).** *Correlation is significant at the 0.05 level (2-tailed).

IL: interleukin; TNF: tumor necrosis factor; NEFL: neurofilament light polypeptide; IFN: interferon.

**CONCLUSIONS**

We successfully established a whole-body exposure system of natural exposure to respirable PM (i.e., PM₁₀) to investigate chronic *in vivo* impacts. To assess the exposure conditions, the levels of PM₁₀ were measured on-line. Our previous findings showed that chronic exposure to this pollutant caused short-term memory loss, brain edema, and neuroinflammation in rats. Microglia activation was also observed following exposure. The present study further demonstrates that systemic neuroinflammatory and immune responses occur in rats after chronic exposure to PM₁₀. Our results suggest that chronic exposure to PM₁₀ may play a larger role than gaseous pollutants in triggering neurotoxicity. Critically, NEFL polypeptide in serum may be a biomarker for this damage.

**ACKNOWLEDGMENTS**

This study was funded by the Ministry of Science and Technology of Taiwan (MOST 108-2314-B-038-093). Authors wholeheartedly thank Miss Yi-Ying Chen, Mr. Zhe-Wei Lin and Mr. Xiao-Yue Chen for technical assistance during this project.

**DISCLAIMER**

The authors declare that they have no conflicts of interest.

**SUPPLEMENTARY MATERIAL**

Supplementary data associated with this article can be found in the online version at http://www.aaqr.org.

**REFERENCES**


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Received for review, August 8, 2019

Revised, February 6, 2020

Accepted, March 19, 2020