DOCTORAL THESIS

Causes of neurological disorders: associations of pm2.5 exposure and intestinal disorders

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Abstract

Objective The aims of this project were to (a) perform a systematic review and meta-analysis of the associations between multiple neurological disorders (or neurological diseases) and potential influencing factors, including the association between fine particulate matter ($PM_{2.5}$) and intestinal dysfunction, and (b) investigate the mechanisms and toxicological effects of $PM_{2.5}$ exposure in the brain and intestines using a mouse model of Alzheimer's disease (AD).

Design A systematic review and meta-analysis was conducted to assess the risks of $PM_{2.5}$ exposure, as manifested by the incidence of exposure-associate neurological disorders or intestinal dysfunction. An APP/PS1 transgenic mouse model for AD was used to study the brain damage resulting from $PM_{2.5}$ exposure, and the miRNA/mRNA regulatory mechanisms contributing to this damage. The inflammatory injuries and bacterial community changes in the intestines of AD mice exposed to $PM_{2.5}$ were also investigated.

Data sources Articles for systematic review and meta-analysis were obtained by searching PubMed and China National Knowledge Infrastructure (CNKI), which were published for more than ten years. Animal experiments were conducted at Shanxi University of Taiyuan in China, and toxicological tests were performed according to the stipulated methods and protocols.

Review and experimental methods Data on the risks of incidence of neurological disorders associated with the environmental factor ($PM_{2.5}$) and biological factors (intestinal disorders and bacteria) were obtained, and random- or fixed-effects models (depending on the I^2 value) were used to pool the odds ratios (OR) with the 95% confidence intervals (CI) from individual studies. In the animal experiments, mice were divided into four groups of five animals per group, as follows: normal control mice in filtered air, AD mice in filtered air, normal control mice in $PM_{2.5}$ air, and AD mice in $PM_{2.5}$ air. $PM_{2.5}$ mice were exposed to ambient $PM_{2.5}$ in a whole-body inhalation exposure device for 8 weeks in Taiyuan, China. Well-established

methods were used to explore the toxicological mechanisms by which $PM_{2.5}$ exacerbated brain damage in AD mice, namely open-field testing, enzyme-linked immunosorbent assay (ELISA), real-time quantitative RT-PCR, hematoxylin-eosin (HE) staining, and transmission electron microscopy (TEM). Brain damage and related biomarkers in the brains were measured, and miRNA and mRNA profiles were detected using high-throughput sequencing methods. The signaling pathways of miRNAs or mRNAs were predicted and summarized, and specific miRNAs and mRNAs were screened to explore the possible regulatory mechanisms of $PM_{2.5}$-induced brain damage in AD mice. Intestinal and fecal samples from these mice were also subjected to 16S rRNA gene sequencing. HE staining, ELISA, and metagenome bacterial diversity analyses were performed to investigate the effects of $PM_{2.5}$ inhalation on intestinal tissue damage, inflammatory responses, and changes of bacterial diversity and communities in AD mice.

Results Long-term $PM_{2.5}$ exposure has been associated with increased risks of stroke, dementia, AD, autism spectrum disorder (ASD), and Parkinson's disease (PD) in humans, with the risks of ischemic and hemorrhagic stroke being higher than that of stroke in general. Furthermore, a relatively higher risk of stroke has been observed in heavily polluted countries compared to less polluted countries. It is known that some intestinal disorders and related problems such as constipation, inflammatory bowel disease, irritable bowel syndrome, small intestinal bacterial overgrowth, and diarrhea significantly increase the risks of developing AD or PD. For example, the risk estimates of *Helicobacter pylori* infection were significantly associated with AD and PD. From another angle, preliminary animal experimental results showed that $PM_{2.5}$ promoted brain morphological damage and decreased spatial exploration ability in AD mice, and was concomitant with increases in the concentrations of amyloid-β-42, acetylcholinesterase, tumor necrosis factor-α, and interleukin-6 and decreases in the concentrations of choline acetyltransferase. High-throughput sequencing and bioinformatics analyses revealed that miRNAs and mRNA had differential expression profiles subsequent to $PM_{2.5}$ exposure, which

suggested that these species are involved in the molecular regulatory mechanisms and possible signal pathways of $PM_{2.5}$-aggravated brain injury in AD mice. These $PM_{2.5}$-aggravated brain injuries were correlated with pathological intestinal injury, inflammatory responses, and changes in bacterial diversity in the intestines and feces of $PM_{2.5}$-exposed AD mice, and decreases in predominant bacteria were identified. These data will assist in delineating the ability of $PM_{2.5}$ exposure to induce pathological changes in the brain and gut tissue via the brain-gut axis and thereby aggravate AD.

Conclusions A systematic review and meta-analysis showed that there is a significant association between $PM_{2.5}$ exposure and the occurrence of stroke, dementia, AD, ASD, and PD, and a strong association between intestinal disorders and the presence of certain bacteria and the development of AD and PD. $PM_{2.5}$ (environmental factors) and intestinal disorders accompanied by changes in bacterial diversity (internal biological factors) appeared to be the two most important factors that increase the risk of developing neurological disorders. Experimental animal data showed that $PM_{2.5}$ potently damaged the brain and intestines of AD mice, and that the toxicological mechanisms of this $PM_{2.5}$-mediated brain injury led to morphological changes, inflammation, and perturbation of miRNA/mRNA regulation in the brain. These data suggest that $PM_{2.5}$ inhalation also have modulatory effects on the abundance and diversity of intestinal bacteria in AD mice. The findings of this study have clarified positive relationships between environmental and biological factors and neurological disorders and have elucidated the potential mechanisms by which $PM_{2.5}$ may mediate the initiation or exacerbation of AD.

Keywords: Neurological disorders, Alzheimer's disease, $PM_{2.5}$, Intestinal disorders, Meta-analysis, Brain, Intestine, Toxicological effects

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