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Particulate matter increases beta-amyloid and activated glial cells in hippocampal tissues of transgenic Alzheimer's mouse: Involvement of PARP-1

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ABSTRACT

Exposure to air pollutants, such as particulate matter (PM), has been implicated in neurodegenerative disorders including Alzheimer's disease (AD). However, direct effects of PM on production of β-amyloid (Aβ), a key pathogenic molecule in AD, and its underlying mechanism are still elusive. Given PM's potential to induce oxidative stress in other tissues, we hypothesized that poly(ADP-ribose) polymerase (PARP-1) might be involved in PM-induced neurotoxicity. To address this, we used an *ex vivo* model of AD, the organotypic hippocampal slice tissue culture from old (12-14 months-of-age) triple transgenic 3xTg-AD mice. First, we observed that fine PM (aerodynamic diameter < 4 μm) can dose-dependently activate PARP-1 and decrease NAD^+ levels in Neuro2A cells. PARP-1 activation did occur under concentrations of PM which did not affect cell viability. Next, we observed that direct treatment of PM increased Aβ levels and activated glial cells in the *ex vivo* hippocampal tissues of 3xTg-AD mice. PM-induced glial activation was most prominent in CA1 region of the hippocampal tissue. Notably, we found that pharmacological inhibition of PARP-1 reversed both PM-induced Aβ increase and glial activation, arguing the possible involvement of PARP-1 in PM-induced AD pathogenesis. Our findings suggest that PARP-1 might be a potential molecular target, responsible for mediating negative effects of PM on the brain. Modulating PARP-1 activity could be a promising approach to prevent or alleviate PM-related environmental neurotoxicity which could initiate AD pathogenesis.

1. Introduction

Air pollution contributes to various types of illnesses including not only respiratory, but also cardiovascular, metabolic and cognitive disorders, and thus is associated with mortality: approximately 40,000 deaths each year in the United Kingdom according to a recent report [1]. Therefore, in addition to efforts reducing generation of air pollutants, it would be crucial to investigate their pathogenic mechanisms to facilitate a secondary preventive measure.

Particulate matter (PM) refers to complex mixture of inorganic and organic particles, which are extremely small in size and hazardous to human health. It is classified by aerodynamic diameter as the coarse (2.5–10 μm), fine (<2.5 μm), and ultrafine (<0.1 μm) particles. Once inhaled, PM can infiltrate, depending on their aerodynamic size, into circulation and thus affect various organs including the brain [2,3].

Epidemiological studies have indicated that PM exposure is associated with an increased risk of Alzheimer's disease (AD) [3–5]. In a *post-mortem* pathological examination, hallmarks of AD pathology such as β-amyloid (Aβ), hyperphosphorylated tau, and neuroinflammation were already identified in the brain tissues of young individuals who had lived in cities with high levels of air pollution [6,7]. This raised a provoking possibility that PM may initiate pathogenic cascades of AD even at young age. Biological studies have also provided evidence that PM exposure can increase β-amyloid (Aβ) and plaque formation, with underlying mechanisms

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unidentified [5,8].

Despite this potentially close association between PM exposure and AD pathogenesis, only one recent study has examined direct effects of PM on Aβ metabolism through an *in vitro* setting [5]. Furthermore, it has remained unknown which molecule mediates PM's actions on AD pathology. Based on previous findings showing that PM induces oxidative stresses, we hypothesized that poly(ADP-ribose) polymerase-1 (PARP-1) might be involved in PM-induced AD pathology. Our research question was straightforward: Does PM directly affect Aβ levels? If so, is PARP-1 involved in such process? To address these questions, we used an *ex vivo* model of AD, organotypic hippocampal slice culture (OHSC) of old 3xTg-AD mice [9], and found that PM (diameter < 4 μm) directly increases Aβ and neuroinflammation, both of which were suppressed by pharmacological inhibition of PARP-1, arguing that PARP-1 is responsible for PM-induced AD pathogenesis.

2. Materials and methods

2.1. Animals

Twelve to 14-month-old triple transgenic (3xTg-AD) mice harboring the familial AD mutations: APPSwe, PS1/M146V, and tauP301L (The Jackson Laboratory, Bar Harbor, ME) were used in this study. Animals were maintained under standard laboratory conditions of 12/12 h light-dark cycles and food and water *ad libitum*. All animal experiments were approved by the Committee for the Care and Use of Laboratory Animals at Yonsei University Health System and performed in accordance with the National Institute of Health guidelines for the Care and Use of Laboratory Animals.

2.2. Reagents and antibodies

Fine PM (Standard Reference Material 2786; <4 μm) was purchased from National Institute of Standards & Technology (Gaithersburg, MD), and PARP-1 inhibitor (veliparib) was from Selleck chemicals Inc. (Houston, Tx). Antibodies to poly(ADP-ribose) (PAR) (1:5000) was from Enzo Life Science, Inc. (Farmingdale, NY), Rabbit anti-PARP-1 polyclonal antibody, and antibodies to GAPDH (1:1000) and GFAP (1:100, sc-6170) were from Santa Cruz Biotechnology (Santa Cruz, CA). 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) was from Promega (Madison, WI). 4′,6-diamidino-2-phenylindole (DAPI) from Thermo Fisher Scientific, Waltham, MA.

2.3. Cell culture

The mouse neuroblastoma cell line Neuro2A was purchased from ATCC (Manassas, VA). Cell line were maintained in Dulbecco's Modified Eagle's Medium (GE healthcare, South Logan, UT) with 10% fetal bovine serum (GE healthcare) in a humidified incubator containing 5% CO_2, at 37 °C. Cells were plated in 6-well plates.

2.4. Western blot analysis

Neuro2A cells were washed with phosphate-buffered saline (PBS) and lysed with ice-cold RIPA buffer (Thermo Fisher Scientific) at 4 °C. After centrifugation, equal amounts of protein were loaded onto 4–12% bis-Tris gel or 10% Tris-glycine gel. Following electrophoresis, proteins were transferred to nitrocellulose membranes (GE healthcare) using wet transfer systems (Mighty Small Transphor, GE healthcare). Membranes were incubated with primary antibodies at 4 °C overnight and then HRP-labeled secondary antibodies at room temperature for 1 h. Protein bands were detected with Amersham ECL western blotting reagent (GE Healthcare) using LAS mini system (Fuji Film, Tokyo, Japan) and densitometrically analyzed with ImageJ software (1.47v; http://rsbweb.nih.gov/ij/, National Institutes of Health, Bethesda, MD).

2.5. Cell viability assay

Cell viability was assessed by the MTS assay. Neuro2A cells seeded in 96 wells plates at a concentration of 1×10^4 cells/ml were exposed to a range of concentration of PM. After 24 h, MTS was added to the medium. The optical density of each well was measured using a microplate reader (VersaMax™; Molecular Devices, San Jose, CA) at 492 nm.

2.6. NAD/NADH measurement

The NAD+ and NADH contents was measured with whole cell extracts of Neuro2A cells using a commercial NAD/NADH quantification kit (Biovision, Mountain View, CA) according to the manufacturer's instructions.

2.7. OHSC of 3xTg-AD brain and drug treatment

OHSC was performed with serum-free medium as described [9,10]. Immediately after decapitation of mice, hippocampi were rapidly dissected out in the chilled dissection medium composed of hibernate A (BrainBits, Springfield, IL), 2% B27 supplement, 2 mM L-glutamine by GlutamMax and antibiotic-antimycotics (all from Invitrogen, Carlsbad, CA). Subsequently, slices (300 μm thick) were cut coronally with a manual tissue slice chopper (#390610, Vibratome company, Saint Louis, MO). The slices were carefully separated under stereo microscope in fresh chilled dissection media (DM) and transferred onto sterile membrane inserts (PICM0RG50; Millipore, Billerica, MA) in 6-well plates. Slices were kept at 37 °C in a humidified 5% CO2 atmosphere. Serum free medium were used, which contains Neurobasal A with 2% B27, 2 mM L-glutamine, and antibiotic-antimycotics until days *in vitro* (DIV) 4. Antibiotic-antimycotics was removed from DIV 5. The whole medium was changed firstly at DIV 1 and changed by half 3 times a week thereafter.

On DIV 11, slices were randomly allocated into 4 groups: control, PM, PM plus PARP-1 inhibitor, and PARP-1 inhibitor. We applied 30 μg/ml PM or 10 nM veliparib into membrane-containing wells for 24 h.

2.8. Quantification of Aβ by ELISA

In each well, 6 slices were cultured on the membrane for 12 days. On DIV 12, the slices and culture media were collected 24 h after PM and drug treatments. For measuring Aβ in the tissue, we homogenized slices in RIPA lysing buffer containing 50 mM Tris-HCl, 150 mM NaCl, 1% Triton X-100, 2 mM EDTA, 0.1% SDS, 0.5% Sodium deoxycholate and protease inhibitor for 15 min. The homogenized slices were centrifuged and the supernatant were used for ELISA procedure. The media samples were concentrated with Amicon Ultra-0.5 mL centrifugal filters (Millipore), because of low quantity of Aβ in the media. ELISA assay was done with human Aβ (1–42) ELISA kit (EZHS42; Millipore). Tissue Aβ level was normalized by protein level quantified with BCA protein assay kit (Thermo Fisher Scientific).

2.9. Propidium iodide (PI) uptake assay

We conducted the PI uptake assay to assess cell damage in OHSC of 3xTg-AD upon 24 h PM treatment. Slices were incubated with PI (10 μg/ml; Sigma-Aldrich) in culture medium for 30 min. The

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