High Vulnerability of Oligodendrocytes to Oxidative Stress Induced by Ultrafine Urban Particles

Ji Young Kim [1], Jin-Hee Kim [2], Yong-Dae Kim [3,4] and Je Hoon Seo [1]

[1] *Department of Anatomy, Chungbuk National University College of Medicine*
[2] *Department of Biomedical Laboratory Science, college of Health Science, Cheongju University*
[3] *Department of Preventive Medicien, Chungbuk National University College of Medicine*
[4] *Chungbuk Regional Cancer Center, Chungbuk National University Hospital*

Introduction

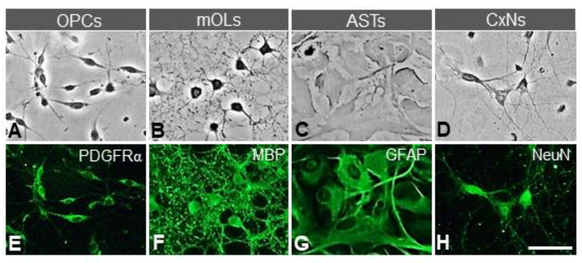
Ultrafine particulate matters (PMs) are commonly defined as air pollution particles smaller than 0.2 μm in diameter. Ultrafine PMs have a significantly greater impact on humans than coarse (PM10) and fine PMs (PM2.5), which have larger particle sizes. Unlike coarse and fine PMs, airborne ultrafine PMs can reach the lungs, since they are not filtered by the nose, trachea, or bronchi during normal breathing. In addition, recent studies have shown that the accumulation of ultrafine PMs in alveoli increases the risk of pneumonia and lung cancer. Worse, ultrafine PMs can pass through the blood-air barrier of the lung and enter the bloodstream, which can lead to various cardiovascular diseases. Furthermore, since it has been shown that ultrafine PMs pass through the blood-brain barrier of 14-week-old Fisher-344 rats, it has been argued that they may also be linked to human brain diseases.

Experimental evidence has indicated that ultrafine PMs in the brain may increase reactive oxygen species (ROS) production and subject brain cells to damage induced by oxidative stress, leading to Parkinson's disease and Alzheimer's disease. Recent cohort studies have demonstrated a strong correlation between air pollution and the risk of **multiple sclerosis (MS)** caused by demyelination of white matter. Moreover, severe air pollution in Tehran, Iran was found to have caused a surge in the number of MS patients. Another study in Lombardy showed a statistically significant correlation between PM concentration and MS-related hospitalization. These previous findings suggest that ultrafine PMs directly affect both myelin and oligodendrocytes (OLs), myelin-forming cells in the central nervous system. However, to date there have been no empirical studies of this relationship using experimental animals and little is known about the underlying mechanism by which ultrafine PMs damage OLs and cause MS.

Depending on the degree of differentiation, OLs are generally classified as oligodendrocyte progenitor cells (OPCs), premyelinating OLs, or mature myelinating OLs (mOLs). Each type of OL lineage cell has a unique shape and phenotypical antigenicity. In the adult brain, the majority of OL lineage cells are mOLs, although a few adult OPCs remain. Both types of cell play crucial roles in remyelination. Since OL lineage cells have lower concentrations of antioxidant enzymes—e.g., glutathione peroxidase and catalase—than other types of brain cell, they are highly vulnerable to oxidative stress. In this study, we evaluated the effect of exposure to ultrafine PMs on OPCs and mOLs via both in vitro and in vivo experiments. We determined whether exposure to **ultrafine urban particulate matters (uf-UPs) as ultrafine PMs** produced ROS and damaged brain cells isolated from neonate rats. We also examined extent of the damage to cerebellar white matter OPCs and mOLs of Balb/c mice after 4 weeks of exposure to uf-UPs by nasal instillation.

Materials & Methods

1. Glia & primary cortical neuron cell culture
   - Shaking incubation : 200rpm, 37℃ → oligodendrocyte precursor cells (OPCs), mature oligodendrocytes (mOLs) and astrocytes (ASTs) isolation from SD rat (P1)
   - Culture media: OPC media for OPCs, mOL media for mOLs, 10S DMEM for ASTs and Neurobasal media for cortical neurons (CxNs)

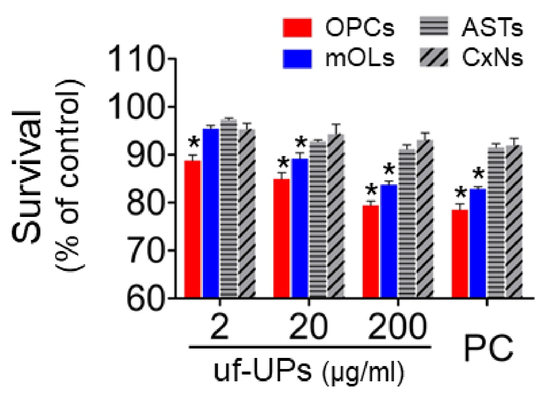


Identification of neural cells isolated from neonate rat brains. (A–D) Morphology visualized by phase contrast microscope. (E–H) Expression of specific antigens. Scale bar = 50 μm.

2. Preparation of uf-UPs and exposure to Cells
   - uf-UPs (NIST 1648a) concentration: 2, 20 and 200 ug/ml
   - Exposure condition: 24h at 37℃ in 5% CO_2 incubation
3. Cell viability analysis
   - MTT assay for cell survival & Hoechst stain for detection of dead cell
4. Measurement of ROS production
   - DCFH-DA assay & DHE stain
5. Measurement of Total antioxidant capacity
   - CUPRAC assay
6. Experimental animals and exposure of uf-UPs
   - P7 weeks Balb/c mouse (20-22g, n=6)
   - uf-UPs exposure: 20ul of the uf-UP solution (0.4mg/ml) for 4 weeks → Instilled into the nasal cavity
7. Immunofluorescence and Fluoro-Jade B staing
   - Cell specific markers: PDGFRa (OPCs), MBP and CA-II (mOLs), GFAP (AST), NeuN (CxN), NG2 (adult OPCs)
   - Fluoro-Jade B (0.004%) → RT incubation for 20 min
8. Statistical analysis: Prism 5 (GraphPad), $p < 0.05$

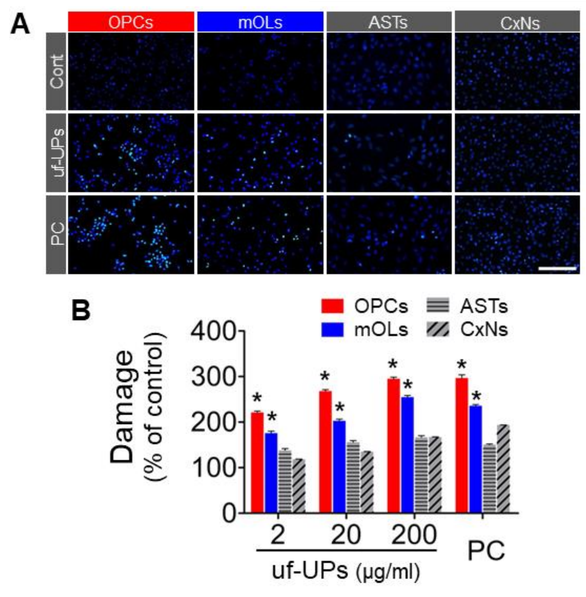
Results

1. Cell survival after uf-UP exposure



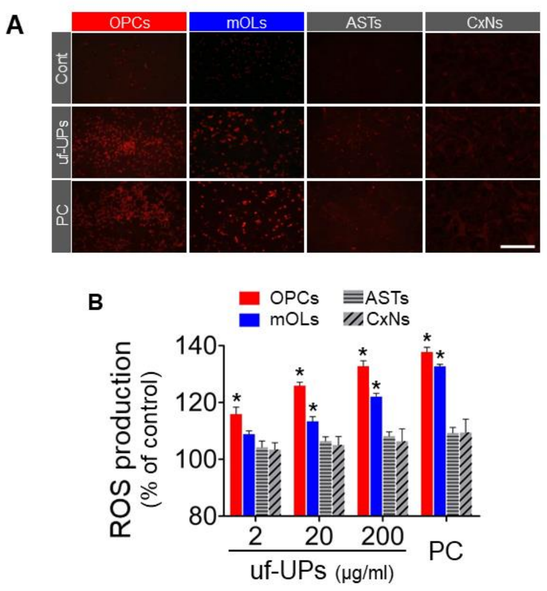
MTT assay. Survival rates of OPCs and mOLs are significantly lower than those of ASTs and CxNs from 2 and 20 μg/mL uf-UPs, respectively. Hydrogen peroxide (H_2O_2, 100 μM) is used as a positive control (PC). Note that the survival rate of each cell type at 200 μg/mL uf-UPs is similar to the rate at 100 μM of hydrogen peroxide. Data are expressed as percentage of the control and represent mean ± SEM (n = 8). * $p < 0.05$ vs. ASTs and CxNs.

2. Cell damage after uf-UP exposure



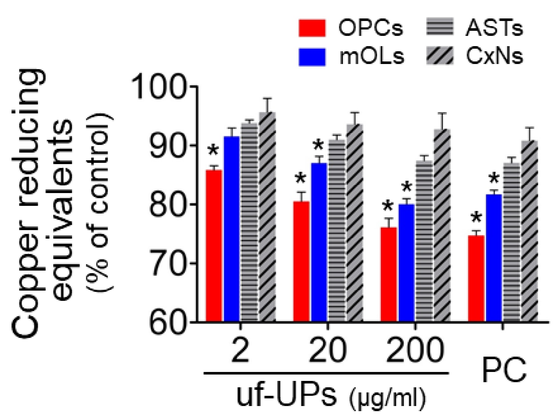
Hoechst staining. (A) Hoechst staining. A large number of damaged OPCs and mOLs are visible after uf-UP exposure (200 μg/mL). (B) Comparison of damaged cells under various uf-UP concentrations. A significantly greater proportion of OPCs and mOLs than ASTs and CxNs are damaged by more than 2 μg/mL uf-UP exposure. Hydrogen peroxide (H_2O_2, 100 μM) is used as positive control (PC). Note that the damage rate of each cell type at 200 μg/mL uf-UPs is similar to the rate at 100 μM hydrogen peroxide. Data are expressed as percentage of the control and represent mean ± SEM (n = 8).
* $p < 0.05$ vs. ASTs and CxNs.
Scale bar = 200 μm.

3. Reactive oxygen species production after uf-UP exposure



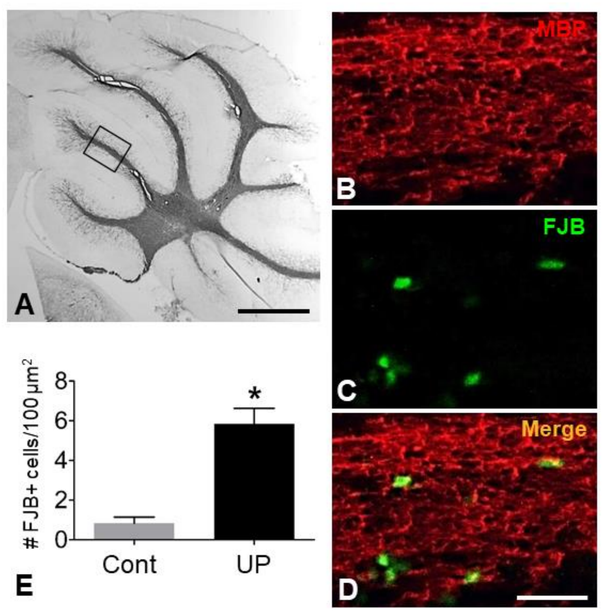
DHE staining and DCF assays. (A) DHE staining. Large amounts of ROS are produced in OPCs and mOLs after uf-UP exposure (200 μg/mL). (B) DCF assay. The amounts of ROS produced by OPCs and mOLs are significantly higher the ROS levels produced by ASTs and CxNs after exposure to more than 2 and 20 μg/mL uf-UPs, respectively. Hydrogen peroxide (H_2O_2, 100 μM) is used as a positive control (PC). Note that ROS production in each cell type at 200 μg/mL uf-UPs is similar to the ROS production level at 100 μM hydrogen peroxide. Data are expressed as percentage of the control and represent mean ± SEM (n = 8). * $p < 0.05$ vs. ASTs and CxNs.
Scale bar = 200 μm.

4. Total antioxidant capacity after uf-UP exposure



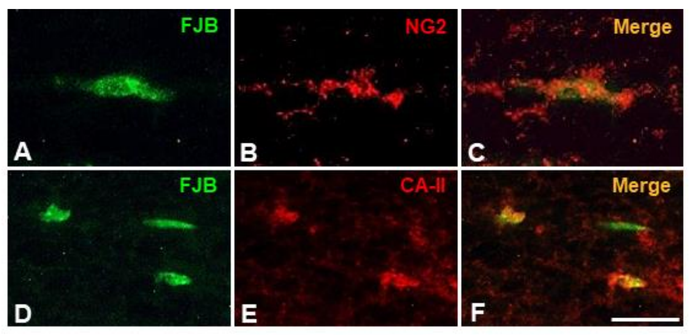
Total antioxidant capacity (TAC) assay. The TACs of OPCs and mOLs are significantly lower than those of ASTs and CxNs for more than 2 and 20 μg/mL uf-UP exposures, respectively. Hydrogen peroxide (H_2O_2, 100 μM) is used as a positive control (PC). Note that the TAC levels of each cell type at 200 μg/mL uf-UPs isare similar to the level at 100 μM of hydrogen peroxide. Data are expressed as a percentage of the control and represent mean ± SEM (n = 8).
* $p < 0.05$ vs. ASTs and CxNs

5. Damaged cells in the cerebellum after uf-UP exposure



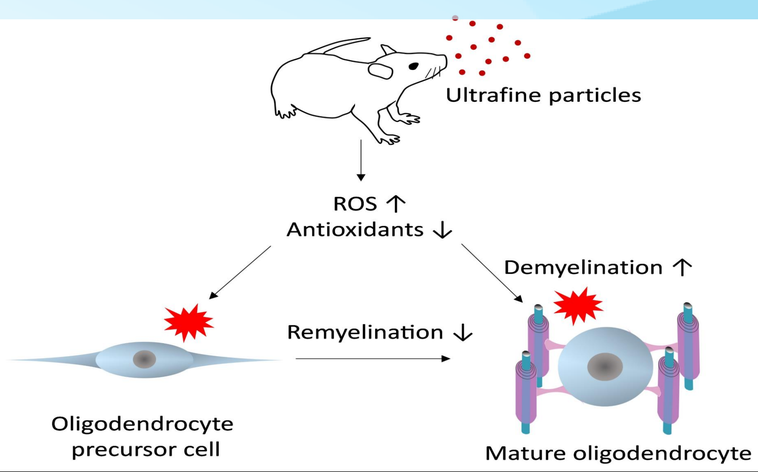
Dead cells in the cerebellar white matter of mice exposed to uf-UPs for 4 weeks. Cerebellar white matter is labeled with an anti-MBP antibody (A). The white matter in the cerebellar folium (shown in the box in panel A) contains Fluoro-Jade B (FJB)-positive cells (B–D). The number of FJB-positive cells in the uf-UP exposure group (UP) is significantly higher than in the control group (E). * $p < 0.05$ vs. control.
Scale bars in A = 500 μm, in D for B–D = 25 μm.

6. Phenotypical identification of damaged cells



FJB-positive cells in the white matter of mice exposed to uf-UPs for 4 weeks. Most FJB-positive cells (green) in the white matter are double-labeled with either NG2 (red), an adult OPC marker (A–C), or CA-II (red), an mOL marker (D–F).
Scale bar = 10 μm.

Summary



- The viability of OPCs and mOLs were lower than astrocytes and neurons after uf-UPs exposure.
- ROS generation in OPCs and mOLs was increased in proportion to the concentration of uf-UPs.
- TAC in OPCs and mOLs is decreased in proportion to the concentration of uf-UPs.
- Damage to adult OPCs and mOLs was observed in the cerebellar white matter of mice exposed to uf-UPs for four weeks.

→ ***Oxidative stress caused by uf-UPs reduces remyelination capacity by causing the death of adult OPCs and induces demyelination by damaging mOLs.***