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Early Responses of Neural Cells (U87) to Particulate Matter Exposure: Cell Viability and Cytokine Concentration

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Abstract: Particulate Matter (PM) has a significant impact on human health and increases the risk of respiratory diseases. PM exposure is linked to various illnesses, including cardiovascular diseases, cancer, neurological diseases, and pregnancy complications, and contributes to both mortality and morbidity globally. PM can also influence the autonomic nervous system. The evidence suggests that the likelihood of developing lung cancer continually rises with the rise in the concentration of coarse PM_{10} (medium aerodynamic diameter less than or equal to 10 μm). However, studies examining the relationship between PM_{10} exposure and brain cancer or gliomas are scarce. In this study, U87 cells were exposed to PM_{10} at three different concentrations and the effects were evaluated by analyzing Lactate Dehydrogenase (LDH) activity, Interleukin-6 (IL-6) expression, and cell viability using a water-soluble tetrazolium assay. The IL-6 concentration in U87 cells varied with PM_{10} concentration and exposure time. PM_{10} did not influence LDH activity in U87 cells at the tested concentrations. These results provide crucial information for future research aimed at understanding the impact of PM on human disease pathology.

Keywords: Cell Viability, Cytotoxicity, IL-6, Particulate Matter, U87 Cells

Introduction

Air pollution is the world's most dangerous and crucial environmental hazard to humans (Kim *et al*., 2020) and is being increasingly recognized as a major public health concern (Cortese *et al*., 2020). Particulate Matter (PM) is the term used for a mixture of solid particles and liquid droplets found in the air (USEPA, 2020). PM has a major effect on human health and increases the risk of developing respiratory diseases (Chaiwangyen *et al*., 2022). Airborne PM has been the focus of extensive research and discussion worldwide for decades (Kelly and Fussell, 2015) and PM pollution was recognized as the ninth major risk factor associated with the global disease burden in 2010 (Suades-González *et al*., 2015). Evidently, the PM concentrations detected in the Republic of Korea are higher than those in other countries such as the United States and Japan (Park *et al*., 2021). PM is classified, according to its size, as ultra-fine (medium aerodynamic diameter less than 0.1 μm, $PM_{0.1}$), fine (medium aerodynamic diameter less than or equal to 2.5 μm, $PM_{2.5}$), and coarse (medium aerodynamic diameter less than or equal to 10 μm, PM_{10}) particles (Mukherjee and Agrawal, 2018; Loaiza-Ceballos *et al*., 2022).

Increased airborne PM concentration has been associated with both morbidity and mortality (Cortese *et al*., 2020); several diseases such as cardiovascular diseases, cancer, and neurological diseases, as well as complications of pregnancy, have been associated with PM exposure (Chaiwangyen *et al*., 2022). The PM also affects the autonomic nervous system (Peters *et al*., 2006). Upon exposure to fine dust, cellular degradation can be caused by various mechanisms and it is related to the induction of oxidative stress and activation of inflammatory reactions (Park *et al*., 2019), which can further lead to acute exacerbation of respiratory and circulatory diseases (Park *et al*., 2019). Several studies have shown that fine dust activates microglial cells that amplify the response to pro-inflammatory stimuli, causing brain cell damage (Morgan *et al*., 2011; Roh *et al*., 2018).

However, studies on the correlation between PM_{10} exposure and brain cancers or gliomas are limited and there is growing interested in the effects of infection and external stressors on nerve cells. Thus, in this study, we

aimed to determine the effects of PM_{10} at varying concentrations and exposure durations on Lactate Dehydrogenase (LDH) activity and Interleukin-6 (IL-6) concentration. This study could help elucidate the effects of external physical stressors such as PM_{10} on U87 cells and lay a foundation for other related studies.

Materials and Methods

Cell Invasion Assay

Cell Culture

We used the U87 cell line, which is a human glioblastoma cell line that is commonly used in brain cancer research (Clark *et al*., 2010).

U87 cells were purchased from the Korean cell line bank (Seoul, Republic of Korea) and cultured in minimum essential Eagle's medium (M4655; Sigma, Gangnam-gu, Republic of Korea) under a humidified atmosphere (5% CO_2) at 37°C. All media were supplemented with 10% Fetal Bovine Serum (FBS) (Corning, Glendale, CA, USA), 100 units/mL penicillin, and 100 units/mL streptomycin. The cells (1×10^4 cells/well) were seeded in 96-well plates and incubated overnight at 37°C under 5% CO_2 for 24 h.

Chemicals

A standard sample of ambient dissolved fine dust (PM_{10}-LIKE; certificate of analysis: ERM-CZ120) from the joint research Centre institute for reference materials and measurements (Geel, Belgium) was used to evaluate toxicity. PM_{10} was diluted in Dulbecco′s phosphate-buffered saline solution (Welgene, Gyeongsan-si, Republic of Korea). The cells were treated with PM_{10} at final concentrations of 10, 300 μg/L, and 1 mg/L for 0 and 24 h.

Water-Soluble Tetrazolium (WST) Assay for Cell Viability

Cell Viability

The effect of PM_{10} on cell viability was determined using the Quanti-MAX WST-8 (tetrazolium salt) Cell Viability Assay Kit (Biomax, Seoul, Republic of Korea). Briefly, 10 μL of WST was added to each well and the cells were incubated at 37°C for 30 min. The absorbance of the samples was measured at 450 nm using a plate reader. RPMI without PM_{10} was used as a negative control and changes in cell viability were determined in triplicate.

LDH Release

LDH release from the cells into the culture medium was monitored using the Quanti-LDH PLUS Cytotoxicity Assay Kit (Biomax). The cytotoxicity of PM_{10} was measured by determining LDH concentration using a colorimetric assay. Aliquots (100 μL) of the cell culture medium were collected from each well and added to microtiter plates. LDH (100 μL) was added to each well and the plates were incubated at 37°C under 5% CO_2 for 30 min then the absorbance of the samples was measured at 490 nm using the flex station 3 multi-mode microplate reader (Molecular Devices, CA, USA). Each experiment was performed in quadruplicate. Cytotoxicity is expressed relative to the basal LDH level of untreated control cells and medium without FBS. The test was repeated three times and the amount of LDH released by the PM_{10} exposed cells is expressed as a percentage relative to that released by the non-exposed cells.

IL-6

IL-6 level was monitored using the Human IL-6 ELISA MAX™ Deluxe Set (BioLegend, CA, USA). One day before conducting the enzyme-linked immunosorbent assay, the Capture Antibody was diluted in 1× Coating Buffer A and then 100 μL of this Capture Antibody solution was added to a 96-well cultured plate with cells. The plate was then sealed and incubated overnight (16-18 h) between 2 and 8°C. Next, 100 μL of diluted Avidin-HRP solution (BioLegend) and 100 μL of TMB substrate solution (BioLegend) were added to each well. After incubating the samples for 15 min, the absorbance was measured at 490 nm using a FlexStation 3 multi-mode microplate reader (molecular devices).

Statistical Analysis

Statistical analyses were performed using a one-way analysis of variance with Tukey's test in SPSS (SPSS version 26.0, IBM, New York) and the results are expressed as mean ± standard error of the mean. The results of LDH activity and IL-6 concentration in U87 cells were analyzed according to the concentration and exposure time of PM_{10}. Post-hoc tests were used to evaluate statistical significance, which was set at $P<0.05$.

Results

U87 Cell Viability Decreased Over Time with Increasing PM_{10} Concentration

We calculated the WST-based cell viability by comparing the absorption of the culture medium of PM_{10}-exposed U87 cells with that of unexposed cells. The average WST concentration at 0 h after adding 10 PM_{10} was 102.3 μg/L in U87 cells, whereas that at 24 h was 96.6 μg/L. After adding 300 μg/L PM_{10} to U87 cells, the WST concentration was 100.5 at 0 h and 94.4 μg/L after 24 h. The average WST concentration after adding 1 mg/L PM_{10} to U87 cells was 98.0 and 95.0 μg/L after 0 and 24 h, respectively (Fig. 1). These results indicate that WST-based viability of U87 cells was reduced over time upon exposure to PM_{10} at concentrations of 10, 300 μg/L and 1 mg/L.

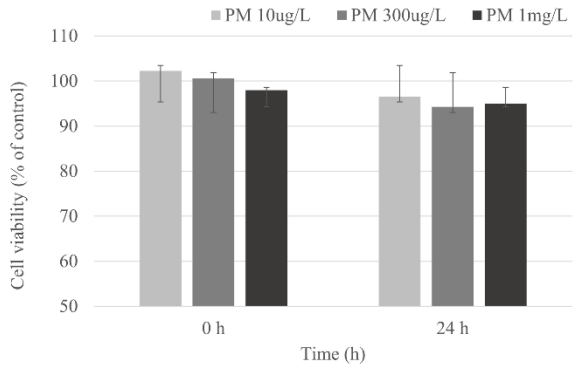

Fig. 1: Effect of PM_{10} at different concentrations on the viability of U87 cells. Light grey bars = U87 cell viability after treatment with PM_{10} 10 µg/L. Grey bars = U87 cell viability after treatment with PM_{10} 300 µg/L. Black bars = U87 cell viability after treatment with PM_{10} 1 mg/L. PM_{10}: Particulate matter

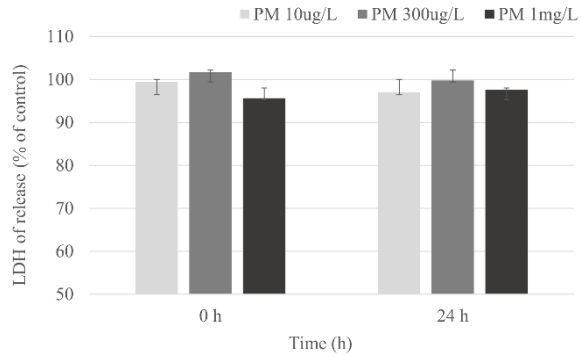

Fig. 2: Effect of PM_{10} at different concentrations on lactate dehydrogenase (LDH) activity in U87 cells. Light grey bars = toxicity in U87 cells exposed to 10 µg/L PM_{10}; grey bars = toxicity in U87 cells exposed to 300 µg/L PM_{10}; and black bars = toxicity in U87 cells exposed to 1 mg/L PM_{10}. LDH: Lactate dehydrogenase; PM_{10}: Particulate matter

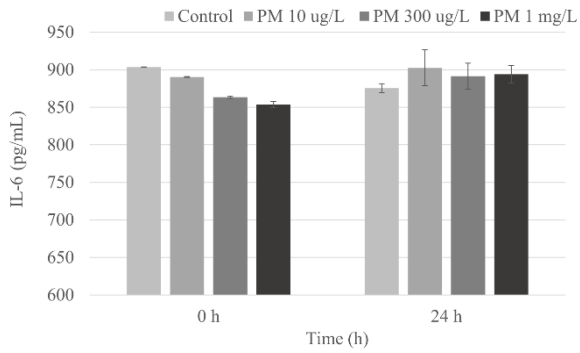

Fig. 3: Effect of PM_{10} on IL-6 production in U87 cells. Light grey bars = IL-6 production in U87 cells exposed to 10 µg/L PM_{10}; grey bars = IL-6 production in U87 cells exposed to 300 µg/L PM_{10}; and black bars = IL-6 production in U87 cells exposed to 1 mg/L PM_{10}. IL-6: interleukin-6; PM_{10}: Particulate matter

PM_{10} Did not Affect LDH Activity in U87 Cells

The level of LDH released by U87 cells was calculated by comparing the absorption of the medium of PM_{10} exposed cells with that of PM_{10} unexposed cells. The LDH activity measured after adding 10 µg/L PM_{10} to U87 cells was 99.5 µg/L after 0 h and 97.0 µg/L after 24 h. After adding 300 µg/L PM_{10} to U87 cells, the LDH activity was 101.7 after 0 h and 99.8 µg/L after 24 h. The LDH activity after adding 1 mg/L PM_{10} to U87 cells was 95.6 and 97.6 µg/L after 0 h and 24 h, respectively (Fig. 2). No difference in LDH activity was observed in U87 cells exposed to PM10 at concentrations of 10, 300 µg/L, and 1 mg/L. These results suggest that the varying concentrations of PM_{10} had no effect on LDH activity at the experimental time points used in this study.

PM_{10} Dose-Dependently Affected Il-6 Concentrations in U87 Cells Over Time

After adding 10 µg/L PM_{10} to U87 cells, the IL-6 level measured at 0 h was 890.17 µg/L, whereas that after 24 h was 902.58 µg/L. After adding 300 µg/L PM_{10} to U87 cells, the IL-6 level after 0 h was 863.29, and that after 24 h was 891.42 µg/L. The IL-6 level in U87 cells 0 h after treatment with 1 mg/L PM_{10} was 853.52 and that after 24 h was 894.19 µg/L (Fig. 3). These results indicate that PM_{10} concentration had a dose-dependent effect on IL-6 level over time in U87 cells. These results also suggest that the higher the PM_{10} concentration, the higher the IL-6 level over time in U87 cells.

Discussion

In this study, U87 cells were treated with PM_{10} at three concentrations to determine its negative effects on the cells. Our results confirmed that U87 cell viability decreased over time with increasing PM_{10} concentration. Lower levels of IL-6 were observed in PM_{10} untreated control cells than in the treated cells; with increasing concentrations of PM_{10}, higher levels of IL-6 were observed in PM_{10} treated cells at all experimental time points. PM_{10} at higher concentrations induced a greater difference in IL-6 expression among the treated and untreated cells over time. Additionally, the differences in LDH activity and IL-6 concentration in U87 cells were dependent on PM_{10} concentration and exposure duration. These findings are consistent with those of a previous study, which reported that ambient air particles affect epithelial cytotoxicity and *in vitro* induction of IL-8 and IL-6 secretion (Frampton *et al*., 1999). LDH is released in response to cell damage, which is reflected by the increase in its baseline level in the extracellular space and blood or other body fluids (Forkasiewicz *et al*., 2020). Thus, LDH is a common marker for cell/tissue damage and helps

identify the type of cell or tissue damaged (Brancaccio *et al*., 2010). It is also commonly used to monitor cytotoxicity and viability in cell culture (Smith *et al*., 2011). IL-6 is an inflammatory cytokine involved in various biological processes, including dysimmune diseases and cancers (Rossi *et al*., 2015). It is produced by many cell lineages, including stromal, hematopoietic, epithelial, and muscle cells (Rossi *et al*., 2015). Furthermore, IL-6 is a multifunctional cytokine that was originally identified as a B-cell differentiation factor involved in the maturation of antibody-producing cells (Cronstein, 2007). Since then, a wide array of additional effects of IL-6 have been identified, including effects on T cells, blood vessels, and neurons (Cronstein, 2007).

Although the risk of developing lung cancer reportedly increases with exposure to increased PM_{10} concentration (García-Cuellar *et al*., 2021), studies on the association between PM_{10} exposure and brain cancers or gliomas are limited. We evaluated LDH activity, cell viability via WST assay, and IL-6 concentrations to determine the effects of PM_{10} exposure on cytotoxicity (Kim *et al*., 2021). LDH release is an indicator of necrosis (Eslaminejad *et al*., 2022; Soletti *et al*., 2010) and the WST assay is a common colorimetric technique used to evaluate cell viability by measuring the activity of intracellular mitochondrial enzymes, an indicator of mitochondrial function in cells (Eslaminejad *et al*., 2022; Soletti *et al*., 2010). Furthermore, the absorption of PM has been shown to elevate the concentration of inflammatory cytokines such as IL-1α, IL-6, IL-10, IL-13, IL-5, and TNF-α (Kim *et al*., 2020). A recent study analyzing cerebrospinal fluid in healthy children showed increased levels of IL-2, IL-6, and MIF in children exposed to high concentrations of air pollution (Kim *et al*., 2020). Hartz *et al*. (2008) showed that exposure to PM resulted in changes in intracellular signaling pathways such as cytokine production and reduced expression of binding proteins (Kim *et al*., 2020), thereby supporting the oxidative and inflammatory effects of air pollution on the progression or development of neurological diseases.

PM can induce the release of cellular inflammatory mediators and cytokines (Peters *et al*., 2006) that further stimulate chemokines, with the involvement of both PM-related endotoxins and water-soluble metals in this process (Shoenfelt *et al*., 2009). PM toxicity contributes to transcription factor activation and induces inflammatory factors, such as nuclear factor kappa-light-chain-enhancer of activated B cells, IL-1, IL-6, IL-8, and cyclooxygenase-2, resulting in the induction of local or systemic inflammation (Chaiwangyen *et al*., 2022; Kim *et al*., 2012). PM can also be absorbed into the lungs through the respiratory tract, inducing the inflammation of lung tissue and activation of T cells that secrete inflammatory cytokines in the brain (Kim *et al*., 2020). Particles absorbed directly through the larynx can induce an inflammatory response in the brain (Kim *et al*., 2020). Thus, air pollutants can affect the cell cycle by inducing cytotoxicity and genetic toxicity (Loaiza-Ceballos *et al*., 2022).

Conclusion

Herein, we have provided substantial evidence for understanding the cytotoxicity of PM_{10} and its effect on inflammation induction; however, our study still has a few limitations. First, we used European-certified CE standard dust as PM_{10}; its effect may differ from that of the fine dust present in the atmosphere. Second, we used an established cell line derived from glioblastoma, which could overestimate the effects of PM on cancer cells compared with those in healthy cells.

Despite these limitations, our study confirmed that the viability of IL-6 concentration in U87 cells varied with PM_{10} concentration and exposure time. Our findings lay a foundation for future studies aiming to elucidate the effects of external substances on cell pathology, including those of heavy metals and viruses. Concise systematic studies with improved methods and precision are required to clearly understand the relationship between external substances and nerve cell responses and to elucidate the processes involved in reducing the adverse effects of air pollution. Moreover, additional research on various environmental factors, such as radiation and ultrafine dust, should be conducted.

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Author's Contributions

Jae-Sik Jeon and Eun Ju Oh: Made substantial contributions to the conception and design of the study. These authors contributed equally to this study.

Qian-Wen Wang and Jae Kyung Kim: Made substantial contributions to the acquisition and analysis of the data.

Ethics

This study was approved by the institutional review board of Dancook University, Republic of Korea (No. 2020-01-011) and was conducted in accordance with the principles of the declaration of Helsinki.

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