American Journal of Biochemistry and Biotechnology

Research Article

Inflammatory Effects of Co-Exposure to Heavy Metals (Cd and Pb) and Respiratory Viruses (229E, OC43, and H3N2) on IL-6 and IL-8 Responses in U87 Glioblastoma Cells

[1]Hyeokjun Yun, [2]Qian-Wen Wang and [2]Jae Kyung Kim

[1]Department of Medical Laser Dankook University Graduate School of Medicine, Republic of Korea
[2]Department of Biomedical Laboratory Science, Dankook University College of Health Science, Chungnam, Republic of Korea

Article history
Received: 11-12-2024
Revised: 15-04-2025
Accepted: 07-05-2025

Corresponding Author:
Jae Kyung Kim
Department of Biomedical Laboratory Science, Dankook University College of Health Science, Chungnam, Republic of Korea
Email: nerowolf@naver.com

Abstract: Co-exposure to heavy metals and viruses can pose threats to cellular health by increasing cytotoxicity and inflammatory cytokine expression. We aimed to elucidate the interactions between these factors in human glioblastoma cells (U87). U87 cells were exposed to influenza A virus (H3N2), coronaviruses OC43 and 229E, and heavy metals Cd and Pb. Cell viability and cytotoxicity were assessed through WST-8 and Lactate Dehydrogenase (LDH) assays. The combined effect of these viruses and metals on Interleukin (IL)-6 and IL-8 inflammatory responses was examined. A high Multiplicity Of Infection (MOI) significantly reduced cell viability, with 0.1 MOI identified as optimal for mild infections. At high MOI levels, 229E and OC43 significantly reduced cell viability, whereas H3N2 caused severe cytotoxicity while preserving cell viability. Elevated LDH levels confirmed cell damage, with OC43 inducing robust IL-8 responses, H3N2 causing moderate IL-6 and IL-8 responses, and 229E triggering a weak IL-6 response. Co-exposure with Cd increased IL-6 and IL-8 levels, indicating pro-inflammatory effects, whereas co-exposure with Pb suppressed IL-8 production. The IL-6 level consistently remained below 800 pg/mL. Conversely, the IL-8 level, which peaked following viral infection, was reduced to below 3000 pg/mL with metal co-exposure. Our findings can aid in formulating healthcare and environmental strategies.

Keywords: Cadmium, IL-6, IL-8, Lead, U87 Glioblastoma Cells

Introduction

The interaction between environmental pollutants viral infections and cellular responses is critical to understanding and managing various health conditions, particularly neurological disorders and certain cancers (Oh *et al.*, 2023a-b). Moreover, it is important to investigate the effects of these external factors on cellular behavior and inflammation to gain insights into disease progression and health outcomes (Oh *et al.*, 2023b).

Human coronaviruses (HCoVs), such as HcoV-229E (229E) and HcoV-OC43 (OC43), as well as the influenza virus (H3N2), exhibit pathogenic profiles that extend beyond respiratory infections (Abdelaziz and Waffa, 2020; Wang and Kim, 2024; Yin and Wunderink, 2018). Their interactions with host cells and their effect on cellular mechanisms pose major challenges in understanding their broad pathological effects (Correia *et al.*, 2020; Desai *et al.*, 2021). These viruses can modulate host immune responses and cellular functions, potentially exacerbating or altering disease outcomes (Abdelaziz and Waffa, 2020; Desai *et al.*, 2021).

Environmental pollutants, particularly heavy metals such as cadmium (Cd) and lead (Pb), are pervasive contaminants commonly found in byproducts of industrial activities including battery production and mining (Boskabady *et al.*, 2018). These metals are well-documented neurotoxicants with significant cytotoxic and immunomodulatory effects. Cd and Pb contribute to oxidative stress, disrupt cellular signaling pathways, and induce inflammatory responses, particularly in neural cells. Previous studies have demonstrated that Cd and Pb exposure is associated with neurodegenerative disorders, impaired immune function, and increased susceptibility to infections (Ganguly *et al.*, 2018; Ge *et al.*, 2019; He *et al.*, 2015). Given their established roles in neurotoxicity and immune dysregulation, Cd and Pb serve as relevant heavy metals for investigating the combined effects of environmental pollutants and viral infections on cellular health.

U87 glioblastoma cells serve as a well-established model for studying neuroinflammatory responses, particularly in the context of viral infections. Although respiratory viruses primarily target airway epithelial cells, certain viruses, including coronaviruses and influenza viruses, have demonstrated the ability to infect or affect neural cells under specific conditions, through neuroinvasion via the olfactory nerve, through the hematogenous spread, or by crossing a compromised blood-brain barrier during systemic infections (Clark *et al.*, 2010; Oh *et al.*, 2023a-b). Documented cases of neuroinvasion with SARS-CoV-2 and H1N1 influenza underscore the relevance of investigating their effects in neural cell models, making U87 cells suitable for exploring potential neuroinflammatory pathways and post-viral neurological complications (Alam *et al.*, 2020; Clark *et al.*, 2010).

In this study, we hypothesized that the combined exposure of U87 neuroglial cells to respiratory viruses and heavy metals can substantially increase cytotoxicity and the expression of inflammatory cytokines. This hypothesis is based on the assumption that viral infection can reduce cell viability and increase cytotoxicity through cellular invasion and replication, thereby causing cell damage (Jakhmola and Jha, 2021; Li *et al.*, 2016; Oh *et al.*, 2023b). Simultaneously, exposure to heavy metals such as Cd and Pb is likely to damage the cell membrane and induce oxidative stress (Al Kahtani, 2020; Wu *et al.*, 2016). The combined effect of these factors is anticipated to exacerbate cellular stress, resulting in heightened cytotoxicity. Additionally, both respiratory viruses and heavy metals are recognized for their ability to induce inflammatory responses, characterized by increased levels of pro-inflammatory cytokines. In this study, we specifically focused on interleukin (IL)-6 and IL-8 as primary inflammatory markers owing to their established roles in neuroinflammation and immune regulation (Bohmwald *et al.*, 2019; Wang *et al.*, 2022). IL-6 plays a central role in the acute-phase immune response and has been implicated in neuroinflammatory and neurodegenerative disorders. IL-8 functions as a key chemokine that recruits immune cells to sites of infection and inflammation, contributing to the exacerbation of immune responses (Bohmwald *et al.*, 2019). Previous studies have suggested that Cd and Pb exposure can upregulate IL-6 and IL-8 expression, potentially amplifying the inflammatory response in virus-infected cells (Ganguly *et al.*, 2018; He *et al.*, 2015). Thus, by focusing on IL-6 and IL-8, we aimed to elucidate the potential synergistic inflammatory effects of viral infections and heavy metal exposure.

This study was conducted to explore the cytotoxic and inflammatory effects of the viruses 229E, OC43, and H3N2, in conjunction with the heavy metals Cd and Pb, on cellular metabolism. By evaluating cell viability, cytotoxicity, and inflammatory markers, we examined the complex interactions between these factors and their effect on cellular health. The findings of this study could our understanding of the effect of environmental and biological stressors on cellular function and their implications for overall health. Moreover, this integrative approach may provide a foundation for future research and inform public health strategies aimed at mitigating the effects of these critical stressors.

Materials and Methods

Materials

Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) was sourced from Corning (Glendale, AZ, USA). Penicillin/streptomycin solution was purchased from gen-DEPOT (Houston, TX, USA) and trypsin was obtained from Thermo Fisher Scientific (Seoul, Republic of Korea). The Quanti-MAX WST-8 Cell Viability Assay Kit and Quanti-LDH PLUS Cytotoxicity Assay Kit were acquired from Biomax (Guri-si, Gyeonggi-do, Republic of Korea). Reagents for IL-6 and IL-8 quantification were provided by Biotechne (Minneapolis, MN, USA). Absorbance readings were taken using a FlexStation 3 microplate reader (Molecular Devices, San Jose, CA, USA).

Virus Culture

Influenza A (H3N2) virus (subtype A [H3N2] NCCP 43228) was obtained from the National Culture Collection for Pathogens (Cheongju, Republic of Korea). Viral propagation was performed using VeroE6 (for 229E), HCT-8 (for OC43), and MDCK (for H3N2) cell lines, provided by the Korean Cell Line Bank. The infected cells were incubated at 37°C with 5% CO_2 for 24 h. The culture supernatants were centrifuged at 1000× *g* for 6 min, aliquoted, and stored at –80°C. Detailed culture protocols for each cell line are available in the Korean Cell Line Bank guidelines.

Cell Culture

U87 human glioblastoma cells were purchased from the Korean Cell Line Bank and maintained in Eagle's Minimum Essential Medium (Sigma, Gangnam-gu, Republic of Korea) supplemented with 10% fetal bovine serum, 100 units/mL penicillin and 100 units/mL streptomycin. Cells were plated at a density of 1×10^4 cells per well in 96-well plates and incubated for 24 h at 37°C in a humidified incubator with 5% CO_2.

Viral Infection of Cells

U87 cells were infected with OC43 or 229E at A Multiplicity Of Infection (MOI) level of 0.1, 1.0, or 10.0. For infection with H3N2, an MOI level of 0.001, 0.01, or 0.1 was used with trypsin supplementation. Cultures were incubated for up to 24 h post-infection, with uninfected cells serving as controls. The selected MOI

values for OC43, 229E, and H3N2 were based on previous studies demonstrating efficient viral infection and replication in U87 cells within 24 h (Jeon *et al.*, 2023; Oh *et al.*, 2023). The lower MOI range for H3N2, combined with trypsin supplementation, reflects its requirement for proteolytic activation (Oh *et al.*, 2023b). These MOI values were chosen to balance sufficient infection efficiency while avoiding excessive cytopathic effects that could confound data interpretation.

Exposure to Heavy Metals

Cd and Pb standard solutions of 1000 ppm sourced from Kanto Chemical (Tokyo, Japan), were diluted in Dulbecco's phosphate-buffered saline (Welgene, Gyeongsan-si, Republic of Korea). Subsequently, U87 cells were exposed to Cd and Pb at concentrations of 0.01, 0.1, and 1.0 mg/mL for 24 h. The Cd and Pb concentrations (0.01–1.0 mg/mL) were selected based on reported cytotoxic thresholds in neural cell models (Oh *et al.*, 2023). This range is consistent with the levels in environmental and occupational exposure studies (Jeon *et al.*, 2023), ensuring physiological relevance.

Cell Viability Assay (WST-8)

Cell viability was evaluated using the Quanti-MAX WST-8 Cell Viability Assay Kit. After adding the WST-8 reagent to each well, the samples were incubated at 37°C for 30 min. Absorbance was then recorded at 450 nm using a microplate reader. The percentage of viable cells was calculated relative to that of the untreated control, which was set at 100%. Baseline measurements at 0 h served as controls before treatment.

Lactate Dehydrogenase Release Assay

Cytotoxicity was assessed using the Quanti-LDH PLUS Cytotoxicity Assay Kit. The cell culture medium was combined with the LDH assay solution and incubated at 37°C for 30 min. Absorbance was measured at 490 nm to determine LDH release. The percentages represent LDH release relative to the control, with higher values indicating increased cytotoxicity, suggesting extensive cell membrane damage and cell death.

Cytokine Assays

IL-6 and IL-8 levels in cell culture supernatants were quantified using Enzyme-Linked Immunosorbent Assays (ELISAs) (Bio-Techne, Minneapolis, MN, USA). Absorbance at 450 nm was measured with a microplate reader and cytokine concentrations were calculated using standard curves.

Statistical Analyses

U87 cell viability and LDH assay results are presented as mean ± Standard Deviation (SD) from triplicate experiments. IL-6 and IL-8 levels were measured in a single experiment and the results were visualized using GraphPad Prism software (Version 7.00.159; Dotmatics, UK). Statistical significance was determined using a one-way analysis of variance followed by Bonferroni's post-hoc test for multiple comparisons. Results with a p-value of <0.05 were considered statistically significant.

Results

U87 Cell Viability

Table (1) Effect of varying MOI levels of 229E, OC43, and H3N2 on U87 cell viability over 24 h. Data are shown as mean % of control. A decrease in cell viability indicates the cytopathic effects of viral replication. Notably, 229E and OC43 exhibited a moderate reduction in cell viability over time, whereas H3N2 maintained higher viability at lower MOIs but showed a decreasing trend at 0.1 MOI.

U87 cell viability following exposure to different titers of 229E, OC43, and H3N2 viruses was assessed using the WST-8 assay, which revealed varying dose-dependent effects over a 24-hour period (Table 1). Exposure to 229E resulted in a decrease in cell viability at high MOI levels. Notably, OC43 induced a substantial decrease at the highest MOI tested. H3N2 elicited a more stable response, with only slight variations in cell viability across different MOI levels.

Table 1: Cell viability (% of control); MOI, multiplicity of infection

Period		0 h			24 h		
MOI		(0.1)	(1.0)	(10.0)	(0.1)	(1.0)	(10.0)
Virus	229E	82.84	81.43	78.26	90.89	70.14	77.71
	OC43	75.92	82.03	83.91	96.23	85.57	64.69
	H3N2	(0.001)	(0.01)	(0.1)	(0.001)	(0.01)	(0.1)
		105.58	91.11	87.16	101.46	100.52	89.74

LDH Activity in U87 Cells

Table (2) Cytotoxic effect of viral infection on U87 cells, as measured by LDH release (mean % of control) over a period of 0-24 h. LDH release was measured to assess cell cytotoxicity following infection of U87 cells with 229E, OC43, and H3N2 viruses at different MOI levels. The data represent the mean percentage of LDH release relative to that of the uninfected control at 0 and 24 h post-infection. An increase in LDH release indicates greater cell damage or lysis. Notably, OC43 and H3N2 induced a strong dose-dependent cytotoxic effect, while 229E exhibited a moderate effect at higher MOIs.

As shown in Table (2), 229E elicited moderate cytotoxicity at low MOI levels. At a high MOI level (10.0), initial LDH expression was markedly high, suggesting significant cytotoxicity and it decreased over 24 h, likely owing to cell death and reduced cell lysis. OC43 induced substantial cytotoxicity across all MOI levels, with consistently high LDH activity, indicating extensive cell membrane damage and subsequent cell

death over time, similar to 229E. Moreover, H3N2 induced high cytotoxicity, with significant LDH release even at low MOI levels. At 0.1 MOI, the initial LDH level was markedly elevated, indicating severe cell membrane damage, followed by a decrease over 24 h, suggesting significant cell death and reduced LDH release from the remaining viable cells.

Table 2: LDH, lactate dehydrogenase; MOI, multiplicity of infection

LDH release (% of Control)							
Period		0 h			24 h		
MOI		(0.1)	(1.0)	(10.0)	(0.1)	(1.0)	(10.0)
Virus	229E	93.36	106.14	255.65	99.57	101.92	174.06
	OC43	102.74	199.66	510.30	104.89	164.31	331.47
	H3N2	(0.001)	(0.01)	(0.1)	(0.001)	(0.01)	(0.1)
		97.91	203.90	641.28	109.69	152.77	381.94

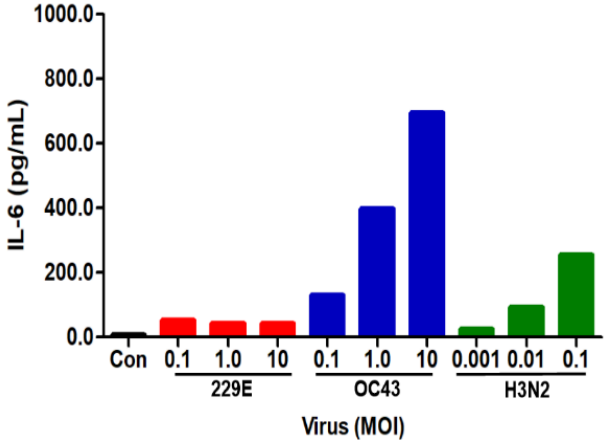

Fig. 1: Effect of viral exposure (229E, OC43, and H3N2) on the IL-6 level in U87 cells. IL-6 level was measured in U87 cells following infection with 229E, OC43, and H3N2 at different MOI levels. The data show a virus-specific IL-6 response, with OC43 infection inducing the highest IL-6 expression in a dose-dependent manner and 229E infection leading to an initial increase followed by a plateau. H3N2 exhibited a moderate IL-6 response relative to OC43. IL, interleukin; MOI, the multiplicity of infection; Con, the uninfected control group

IL-6 Level in U87 Cells Following Viral Exposure

The IL-6 level was assessed in U87 cells exposed to the viruses 229E, OC43, and H3N2 over 24 h (Figure 1). The control group exhibited a baseline IL-6 level of 7.61 pg/mL. However, exposure to 229E resulted in elevated IL-6 level, reaching 52.28 pg/mL at 0.1 MOI; the level plateaued at high MOIs (43.17 at 1.0 MOI and 43.22 pg/mL at 10.0 MOI), indicating a saturation point. This finding suggests a saturation effect, where IL-6 production reaches a maximum threshold beyond which further viral load does not enhance cytokine release (Vilotić *et al.*, 2022). Alternatively, cellular regulatory mechanisms, such as negative feedback loops or immune signaling constraints, might limit IL-6 production at higher MOIs (Ramachandran *et al.*, 2021). OC43 induced a robust, dose-dependent increase in the IL-6 level from 131.03 pg/mL at 0.1 MOI to 695.81 pg/mL at 10.0 MOI. Similarly, H3N2 induced a dose-dependent increase in the IL-6 level from 25.86 pg/mL at 0.001 MOI to 256.59 pg/mL at 0.1 MOI, reflecting a moderate inflammatory response compared with that upon OC43 infection. Overall, OC43 was the most potent inducer of IL-6 production, exhibiting a strong dose-dependent effect. Conversely, 229E resulted in a significant increase in the IL-6 level but lacked a clear dose-response relationship beyond a certain MOI; however, H3N2 elicited a moderate, dose-dependent IL-6 response.

IL-6 Level in U87 Cells Following Cd and Pb Exposure

The IL-6 level in U87 cells was assessed 24 h after exposure to Cd and Pb. The untreated control group exhibited a baseline IL-6 level of 7.61 pg/mL. Upon exposure to Cd, the IL-6 level decreased to 6.41 pg/mL at 0.01 mg/mL, significantly increasing to 344.53 pg/mL at 0.1 mg/mL, followed by a slight decline to 318.37 pg/mL at 1.0 mg/mL. Conversely, Pb exposure resulted in a modest increase in the IL-6 level to 21.52 pg/mL at 0.01 mg/mL, decreasing to 6.02 pg/mL at 0.1 mg/mL, followed by an increase to 21.08 pg/mL at 1.0 mg/mL (Figure 2). These results suggest that Cd-induced considerable cellular stress and inflammation in U87 cells at high concentrations. Although Pb also induced an increase in the IL-6 level, the inflammatory response was notably less intense than that induced by Cd.

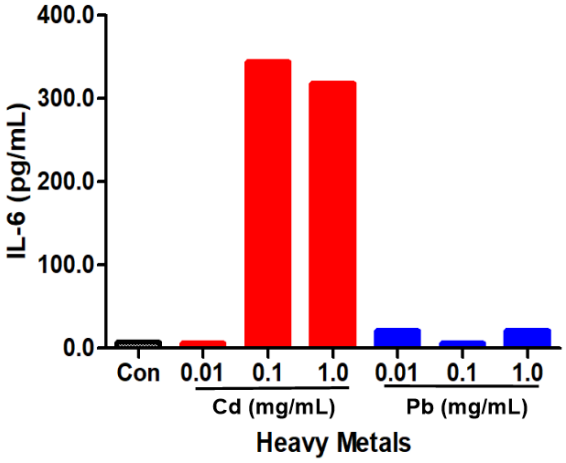

Fig. 2: Effect of Cd and Pb exposure on the IL-6 level in U87 cells. IL-6 level was measured in U87 cells following exposure to different concentrations of Cd and Pb (0.01, 0.1, and 1.0 mg/mL) for 24 h. Cd exposure resulted in a significant dose-dependent increase in IL-6 level, whereas Pb exposure had a minimal effect. IL, interleukin; Con, untreated control group

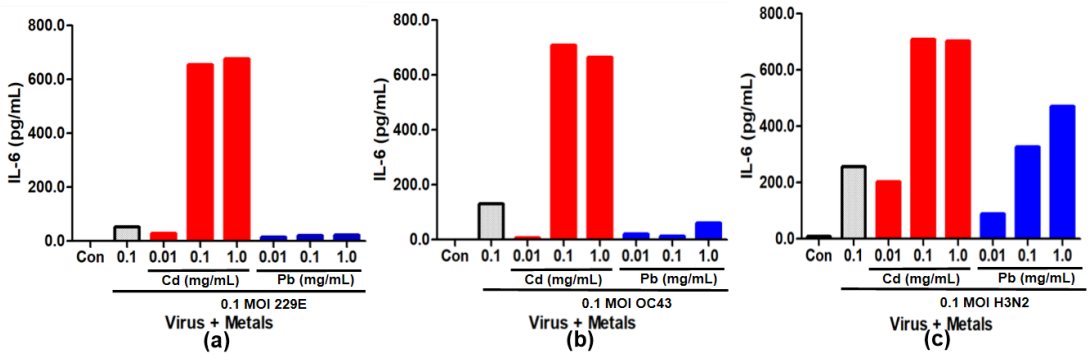

Fig. 3: Effect of Cd and Pb exposure on the IL-6 level in U87 cells treated with the viruses 229E (a), OC43 (b), and H3N2 (c) at 0.1 MOI. IL-6 level was measured in U87 cells co-treated with Cd or Pb at different concentrations (0.01, 0.1, and 1.0 mg/mL) and infected with (a) 229E, (b) OC43, or (c) H3N2 at 0.1 MOI for 24 h. Cd exposure led to a strong dose-dependent increase in IL-6 level following infection with the viruses tested, whereas Pb had a comparatively weaker effect. IL, interleukin; MOI, the multiplicity of infection; Con, the untreated control group

IL-6 Level in U87 Cells Following Co-Exposure to Viruses and Heavy Metals

U87 cells exposed to 229E coronavirus at 0.1 MOI exhibited an initial IL-6 level of 52.28 pg/mL. The addition of Cd reduced this level to 27.34 pg/mL at 0.01 mg/mL; however, this level sharply increased to 653.90 pg/mL at 0.1 mg/mL Cd and remained high at 675.47 pg/mL with 1.0 mg/mL Cd. Pb markedly lowered the IL-6 level to 14.74 pg/mL at 0.01 mg/mL, with a slight increase to 22.26 pg/mL at 1.0 mg/mL in the presence of 229E virus (Figure 3a). U87 cells treated with OC43 at 0.1 MOI exhibited an initial IL-6 level of 131.03 pg/mL. The addition of 0.01 mg/mL Cd decreased this level to 5.39 pg/mL, which subsequently increased to 653.90 pg/mL at 0.1 mg/mL Cd and further increased to 675.47 pg/mL at 1.0 mg/mL Cd. Pb significantly decreased the IL-6 level to 14.74 pg/mL at 0.01 mg/mL, 20.09 pg/mL at 0.1 mg/mL, and 22.26 pg/mL at 1.0 mg/mL in cells treated with OC43 at 0.1 MOI (Figure 3b). U87 cells treated with H3N2 at 0.1 MOI exhibited an initial IL-6 level of 256.59 pg/mL. Cd addition reduced this level to 201.39 pg/mL at 0.01 mg/mL, which subsequently increased to 707.45 and 701.68 pg/mL at 0.1 and 1.0 mg/mL Cd, respectively. Pb significantly decreased the IL-6 level to 89.28 pg/mL at 0.01 mg/mL, which subsequently increased to 325.83 and 469.67 pg/mL at 0.1 and 1.0 mg/mL Pb, respectively (Figure 3c). However, this increase was not as pronounced as that observed with Cd in cells infected with H3N2 at 0.1 MOI. Cd demonstrated a dose-dependent effect on IL-6, resulting in increased inflammatory responses at high concentrations; however, Pb consistently inhibited IL-6 production in the presence of 229E and OC43 but not H3N2.

Effect of Viruses on the IL-8 Level in U87 Cells

The IL-8 level was assessed in U87 cells 24 h after exposure to the viruses 229E, OC43, and H3N2. The control group exhibited a baseline IL-8 level of 9.25 pg/mL. However, exposure to 229E increased the IL-8 level to 540.5 pg/mL at 0.1 MOI, which plateaued at high MOIs (453.25 and 453.75 pg/mL at 1.0 and 10.0 MOIs, respectively). OC43 exposure resulted in the highest IL-8 levels: 1294.5, 3840.25, and 6702.25 pg/mL at 0.1, 1.0, and 10.0 MOIs, respectively, exhibiting a dose-dependent increase. H3N2 also induced a dose-dependent increase in the IL-8 level: 287.5, 939.75, and 2496.75 pg/mL at 0.001, 0.01 and 0.1 MOIs, respectively. Overall, OC43 was most effective in inducing IL-8 production, 229E induced plateauing at high MOIs and H3N2 elicited a moderate, dose-dependent response (Figure 4).

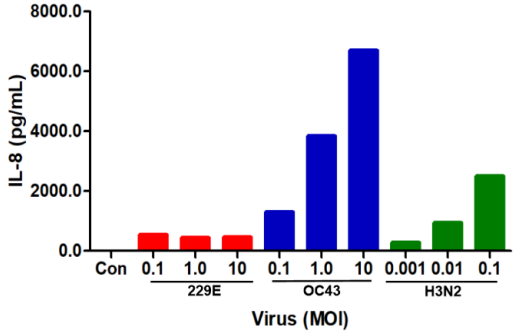

Fig. 4: Effect of viral exposure (229E, OC43, and H3N2) on the IL-8 level in U87 cells. IL-8 level was measured in U87 cells following infection with 229E, OC43, and H3N2 at different MOI levels for 24 h. OC43 induced the highest IL-8 production in a strong dose-dependent manner, whereas 229E elicited only a minimal response. H3N2 led to a moderate increase in IL-8 level compared to OC43. IL, interleukin; MOI, the multiplicity of infection; Con, the untreated control group

Effect of CD and PB Exposure on the IL-8 Level in U87 Cells

The IL-8 level in U87 cells was measured 24 h after exposure to Cd and Pb. The control group exhibited a baseline IL-8 level of 9.25 pg/mL. Upon Cd addition, the IL-8 level increased to 54.25, 95.5, and 148.25 pg/mL at

0.01, 0.1, and 1.0 mg/mL, respectively, indicating a substantial increase with the increase in Cd concentration. Conversely, exposure to Pb caused a slight increase in the IL-8 level, reaching 8.0, 49.25, and 162.25 pg/mL at 0.01, 0.1, and 1.0 mg/mL, respectively (Figure 5). Both Cd and Pb induced IL-8 production in a dose-dependent manner, with high concentrations leading to increased IL-8 level.

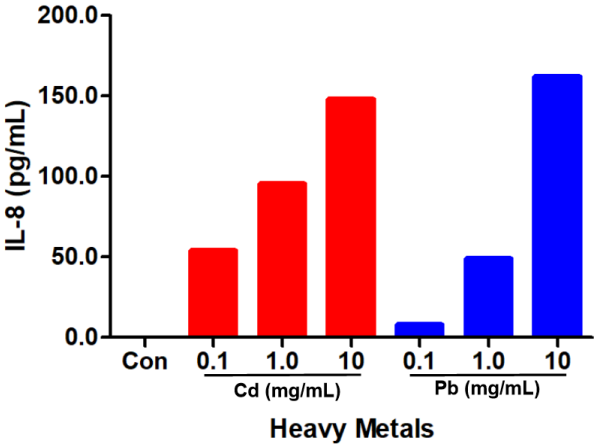

Fig. 5: Effect of Cd and Pb exposure on interleukin-8 (IL-8) level in U87 glioblastoma cells. Cells were treated with increasing concentrations of Cd (0.1, 1.0, and 10 mg/mL, red bars) and Pb (0.1, 1.0, and 10 mg/mL, blue bars), and IL-8 level (pg/mL) was measured in the culture supernatant. The control group value represents the baseline IL-8 level without heavy metal exposure. The results indicate that Cd and Pb induce IL-8 production in a dose-dependent manner, with Cd having a stronger effect at lower concentrations than Pb. IL, interleukin; MOI, multiplicity of infection

Combined Effects of Viral and Heavy Metal Exposure on the IL-8 level in U87 Cells

U87 cells infected with 229E at 0.1 MOI exhibited an initial IL-8 level of 540.5 pg/mL, which decreased to 196.75 pg/mL at 0.01 mg/mL Cd, subsequently increasing with high Cd concentrations (695.5 and 1143.75 pg/mL at 0.1 and 1.0 mg/mL, respectively). Pb addition significantly reduced the IL-8 level (101.75 pg/mL at 0.01 mg/mL and 0 pg/mL at high concentrations). OC43-infected cells exhibited an initial IL-8 level of 1294.5 pg/mL, which decreased to 57.75 pg/mL at 0.01 mg/mL Cd and subsequently increased with increase in Cd concentration (627.25 and 944.5 pg/mL at 0.1 and 1.0 mg/mL, respectively). Pb reduced the IL-8 level to 122.75, 1.5, and 30.25 pg/mL at 0.01, 0.1, and 1.0 mg/mL, respectively. H3N2-infected cells exhibited a high initial IL-8 level (2496.75 pg/mL), which decreased at 0.01 mg/mL Cd (836.25 pg/mL) and increased at 0.1 mg/mL (1614.75 pg/mL), with a slight decrease at 1.0 mg/mL (1517.5 pg/mL). However, Pb induced less pronounced reductions (465.0, 362.75, and 448.25 pg/mL at 0.01, 0.1, and 1.0 mg/mL, respectively). Overall, Cd exhibited a dose-dependent pro-inflammatory effect, whereas Pb consistently inhibited IL-8 production (Figure 6).

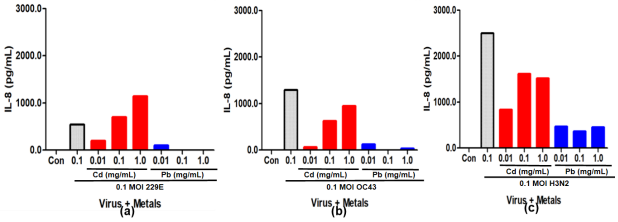

Fig. 6: Effect of Cd and Pb exposure on IL-8 level in U87 glioblastoma cells infected with the human coronaviruses 229E (a) and OC43 (b) and influenza virus H3N2 (c) at MOI of 0.1. IL-8 level (pg/mL) was measured in the culture supernatant. Cells were exposed to increasing concentrations of Cd (0.01, 0.1, and 1.0 mg/mL) and Pb (0.01, 0.1, and 1.0 mg/mL), and the IL-8 level was compared with that of an untreated control (Con). The results indicate that Cd exposure increases IL-8 secretion in virus-infected cells in a dose-dependent manner (red bars), while Pb exposure has a minimal effect (blue bars). The control group values (gray bars) represent the baseline IL-8 level at 0.1 MOI. IL, interleukin; MOI, multiplicity of infection

Discussion

This study provides valuable insights into the differential cytotoxic and inflammatory effects of human coronaviruses (229E and OC43), influenza virus (H3N2), and heavy metals (Cd and Pb) on U87 glioblastoma cells. The findings highlight distinct cellular responses to these pathogens and toxicants, enhancing our understanding of virus-host interactions and environmental toxicity.

The results revealed that both 229E and OC43 coronaviruses reduced cell viability at MOI levels above 0.1, which is typically considered optimal for studying mild infection scenarios in U87 cells. In contrast, H3N2 preserved cell viability consistently across MOIs but demonstrated significant cytotoxicity at high doses. These results align with the notion that U87 cells exhibit optimal viability at 0.1 MOI for both coronaviruses, consistent with previous study observations (Oh *et al.*, 2023b, Zhang *et al.*, 2024). The LDH assay results corroborated substantial cytotoxic effects of all viruses, with OC43 causing the most pronounced damage, consistent with previous research findings (Oh *et al.*, 2023b).

Regarding inflammatory responses measured through IL-6 and IL-8 production, OC43 elicited a stronger IL-8 response than an IL-6 response, whereas H3N2 induced moderate IL-6 and IL-8 responses. Conversely, 229E induced significant but less pronounced IL-6 responses. Previous studies have shown that both 229E and OC43 induce a dose-dependent increase in the IL-8 level in

U87 cells, with OC43 eliciting a more substantial increase (Oh *et al.*, 2023b), supporting our findings. Co-exposure with Cd resulted in consistently elevated IL-6 and IL-8 levels, highlighting the pro-inflammatory effects of Cd. Conversely, exposure to Pb tended to suppress IL-8 production. This observation aligns with the finding of a previous study that the IL-6 level increases notably in response to Cd compared with that in response to Pb (Oh *et al.*, 2023a).

In this study, we examined IL-6 production in U87 cells exposed to coronaviruses (229E, OC43, and H3N2) and heavy metals (Cd and Pb). OC43 induced the highest IL-6 production in a dose-dependent manner, whereas 229E showed saturation at high MOIs, and H3N2 induced a moderate IL-6 level increase. Cd exhibited a biphasic response, with low doses suppressing IL-6 production and high doses significantly increasing it, likely due to oxidative stress. Pb had a more variable effect, generally suppressing IL-6 production except in H3N2-infected cells. Combined exposure to viruses and heavy metals revealed synergistic inflammation with Cd, while Pb mostly inhibited IL-6 production (Figs. 1–3). The study highlights the role of oxidative stress and NF-κB signaling in the regulation of IL-6 levels, warranting further research into these mechanisms (Cheng *et al.*, 2021).

Similarly, IL-8 production in U87 cells was assessed after exposure to the same stressors. OC43 was the most potent IL-8 inducer, exhibiting a strong dose-dependent increase, whereas 229E infection led to a plateau of IL-8 level at high MOIs and H3N2 infection induced a moderate, dose-dependent response. Cd exposure significantly elevated IL-8 levels in a dose-dependent manner, whereas Pb had a milder effect. In virus-infected cells, Cd initially suppressed IL-8 production at low concentrations, but increased it considerably at higher concentrations, indicating that it induces a pro-inflammatory response. Pb consistently reduced the IL-8 level, suggesting an inhibitory effect (Figs. 4–6). These findings suggest that oxidative stress and NF-κB activation play key roles in the regulation of IL-8 levels, warranting further investigation (Chen *et al.*, 2016).

At an MOI of 0.1, infection with 229E and OC43 resulted in enhanced U87 cell viability over 24 h, indicating its suitability for studying mild infection effects. High MOIs resulted in stable or slightly increased viability, suggesting moderate cytotoxicity. Therefore, an MOI of 0.1 is recommended for studying mild 229E and OC43 infections in U87 cells. Conversely, H3N2, owing to its substantial cytotoxicity, was tested at concentrations as low as 0.001 MOI in conjunction with the other coronaviruses.

Recent research has increasingly focused on the combined effects of environmental toxins and viral infections on inflammatory responses. A study investigated how early-life exposure to Cd following Respiratory Syncytial Virus (RSV) infection leads to metabolic reprogramming and heightened inflammation. The findings suggest that Cd exposure exacerbates inflammatory responses initiated by RSV, implicating protein S-palmitoylation in this process (Jarrell *et al.*, 2024). Another study examined the co-exposure effects of urinary polycyclic aromatic hydrocarbons (PAHs) and metals on lung function, revealing that simultaneous elevation in the concentrations of PAHs and metals, particularly Cd, reduced lung function parameters. Systemic inflammation was identified as a mediating factor in these associations, highlighting the complex interplay between environmental pollutants and respiratory health (Wu *et al.*, 2024).

Research focusing on residents near mining and smelting areas in Northwest China demonstrated that co-exposure to Pb and Cd is associated with systemic immune inflammation. Elevated levels of these metals correlated with increased inflammatory biomarker levels, suggesting that combined heavy metal exposure can aggravate immune inflammatory responses (Zhang *et al.*, 2022). These studies underscore the importance of considering combined environmental exposures when assessing inflammatory outcomes, as co-exposure to heavy metals and viral infections can synergistically enhance inflammatory responses, potentially leading to more severe health effects.

Our previous studies on A549 cells, a human lung alveolar carcinoma epithelial cell line, demonstrated similar responses to treatments with human coronaviruses (229E and OC43) and metals (Cd and Pb), showing a significant increase in the IL-6 and IL-8 levels following treatment, accompanied by increased cytotoxicity (Wang and Kim, 2024; Yuan *et al.*, 2019). Notably, both lung and brain cells exhibit similar immune mechanisms in response to viral infections and metal exposure (Wang and Kim, 2024; Wang *et al.*, 2022).

The observed increase in the IL-8 level upon co-exposure to Cd and viruses (229E, OC43, and H3N2), contrasting with the decrease in the IL-8 level with Pb co-exposure, has substantial implications. The increase in the IL-8 level in the presence of Cd suggests that Cd enhances inflammatory responses, given that IL-8 is a key pro-inflammatory cytokine involved in inducing and amplifying inflammatory responses in various cells, including neural cells such as U87 cells (Budden *et al.*, 2019; Oh *et al.*, 2023a-b). Cd induces oxidative stress and cellular damage, leading to the production of inflammatory cytokines such as IL-8 (He *et al.*, 2015). Consequently, co-exposure to Cd and viruses resulted in a heightened inflammatory response within cells. In contrast, the decrease in the IL-8 level with co-exposure to Pb and viruses indicates that Pb likely suppresses inflammatory pathways or diminishes virus-induced inflammatory responses (Oh *et al.*, 2023a; Wang and Kim, 2024).

Cd and Pb exhibit distinct effects on inflammatory responses in viral infections (Cheng *et al.*, 2021; Oh *et al.*, 2023a). Cd generally exacerbates inflammation, whereas Pb tends to attenuate it. This observation suggests that simultaneous exposure to environmental toxins and viral infections can elicit significantly different cellular responses compared with exposure to each factor alone. Understanding the divergent effects of Cd and Pb on the production of inflammatory cytokines such as IL-8 is crucial for studying viral infections and developing treatments in metal-contaminated environments. The differential effects of Cd and Pb on the IL-8 level highlight the intricate interplay between environmental toxins and viral infection mechanisms, emphasizing the importance of evaluating their combined effect on cellular health and disease.

This study has some limitations. First, while U87 glioblastoma cells serve as a well-characterized and reproducible in vitro model for examining neuroinflammatory responses to heavy metal and respiratory virus co-exposure, their relevance to broader physiological contexts remains limited. These cells, though widely used in neuroinflammation research owing to their stable phenotypic characteristics and responsiveness to inflammatory stimuli (Yin *et al.*, 2024), do not fully replicate the complexity of primary neural cells or in vivo systems. Glioblastoma-derived cell lines exhibit distinct molecular and functional properties compared to primary astrocytes or microglia, which are central to neuroimmune interactions (Oh *et al.*, 2023a). This discrepancy raises concerns about the generalizability of our findings, particularly regarding immune signaling and cytokine responses. Future studies should incorporate primary neural cultures or in vivo models to better capture the physiological relevance of metal–virus interactions in the central nervous system. Second, it exclusively focused on Pb and Cd, excluding other heavy metals such as Ag and Ni, as well as pollutants, including microplastics and ozone. This focus might restrict the comprehensiveness of our findings regarding their effects on nerve cells. Additionally, although the study included 229E, OC43, and H3N2, it could be expanded to include other viruses, such as rhinovirus, to ensure a more comprehensive analysis. Future research should encompass a wide array of heavy metals, pollutants, and other viruses to enhance the understanding of their collective effects (Zhang *et al.*, 2024). Third, we focused exclusively on the acute effects of co-exposure, limiting our ability to assess long-term inflammatory or cytotoxic consequences. Results obtained after a 24-hour exposure period may only reflect initial immune activation, without capturing chronic immune dysregulation, sustained oxidative stress, or adaptive cellular responses that may occur with prolonged exposure (Wu *et al.*, 2016; Chen *et al.*, 2016). Chronic exposure to heavy metals and viral infections has been linked to persistent cytokine release, mitochondrial dysfunction, and epigenetic modifications, which could significantly alter immune responses over time. While we aimed to characterize early-stage inflammation, future investigations should incorporate extended exposure durations to determine whether transient inflammatory responses progress into sustained immune activation or cellular adaptation (Chen *et al.*, 2016). Fourth, the observed differences in IL-6 and IL-8 production elicited by distinct viral strains warrant further investigation into their potential correlation with the viruses' replicative capacity in U87 cells. Studies on coronaviruses and flaviviruses have demonstrated that viral replication efficiency influenced IL-6 and IL-8 levels. For example, replication in U87 glioblastoma cells correlated with cytokine induction, highlighting the viral fitness effect on immune responses (Oh *et al.*, 2023b; Flanagan, 2020). Fifth, experiments using UV-inactivated viruses have assessed the viruses' capacity to induce IL-6 and IL-8 production and cytotoxicity (Xu *et al.*, 2023). Despite lacking replication ability, these viruses stimulated immune responses via structural components such as capsid proteins, activating Toll-like receptors and innate immune pathways (Xu *et al.*, 2023). However, the induction of IL-6 and IL-8 production by UV-inactivated viruses was lower than that by replicating viruses owing to the absence of active transcription or replication processes (Xu *et al.*, 2023). These findings clarified whether cytokine responses depend on active viral replication or structural elements. Sixth, the study primarily focused on IL-6 and IL-8 as indicators of inflammation. However, interferons (e.g., IFN-α and IFN-β) and Interferon-Stimulated Genes (ISGs) play essential roles in antiviral immunity and their expression may be modulated by environmental contaminants such as Cd and Pb (Lang *et al.*, 2022; Zhang *et al.*, 2024). To build upon these findings, future investigations should quantify the expression of type I IFN and ISGs (e.g., MX1, OAS1, and ISG15) using advanced methods such as reverse transcription-quantitative polymerase chain reaction and RNA-sequencing (Lee and Ashkar, 2018). Such studies can elucidate the effects of Cd and Pb on IFN-mediated signaling pathways during viral infection and host defense. Finally, future studies should investigate how Cd and Pb affect viral infection-induced signaling pathways, specifically focusing on the Toll-Like Receptor (TLR)-dependent, inflammasome-dependent, NF-κB signaling pathway and RIG-I-Like Receptor (RLR)-mediated responses. These metals may alter TLR signaling by suppressing receptor expression or downstream signaling, potentially impairing the host’s innate immune recognition of viral infections (Lang *et al.*, 2022). Additionally, Cd and Pb could modulate inflammasome activation, affecting the release of pro-inflammatory cytokines such as IL-1β and IL-18, which are crucial to the antiviral response (Lang *et al.*, 2022). The influence of these metals on RLR signaling pathways, which are vital for detecting viral RNA and triggering type I IFN production, should also be

explored, as they may reduce the host's ability to initiate an effective antiviral response (Pervolaraki *et al.*, 2018). Furthermore, one of the primary ways that these heavy metals induce inflammation is through oxidative stress and mitochondrial dysfunction (Ramachandran *et al.*, 2021). Cd and Pb exposure generates Reactive Oxygen Species (ROS), which overwhelm the antioxidant defenses of the cell, leading to the activation of various inflammatory pathways, including the NF-κB signaling pathway playing a critical role in regulating the production of pro-inflammatory cytokines such as IL-6 and IL-8, which are key markers of inflammation and immune response (Ramachandran *et al.*, 2021; Pervolaraki *et al.*, 2018). In U87 glioblastoma cells, exposure to Cd or Pb may activate NF-κB by triggering the degradation of IκB proteins, which normally inhibit NF-κB. Once released, NF-κB dimers translocate to the nucleus, where they promote the transcription of IL-6 and IL-8 (Zhang *et al.*, 2024; Ramachandran *et al.*, 2021). This signaling mediates both innate and adaptive immune responses during viral infections. Moreover, experiments assessing the effect of Cd and Pb on viral replication, using viral load quantification and pathway inhibitors, will provide insights into how these toxins alter virus-host interactions, by either directly affecting viral replication or modulating immune responses, thereby offering a comprehensive understanding of their role in viral pathogenesis (Lang *et al.*, 2022).

Given the growing recognition of air pollution as a major public health concern, examining the effects of particulate matter (PM_{10}) alongside metals and respiratory viruses is essential (Liu *et al.*, 2019). Studies have shown that PM_{10} enhances IL-6 and IL-8 activities and cytotoxicity in U87 and A549 cells (Jeon *et al.*, 2023; Wang *et al.*, 2022). Future research should investigate the combined effect of these pollutants on human cells.

Conclusion

This study demonstrated the differential inflammatory and cytotoxic effects of human coronaviruses (229E and OC43), influenza virus (H3N2), and heavy metals (Cd and Pb) on U87 glioblastoma cells. The findings indicate that OC43 elicits the strongest IL-8 response, whereas H3N2 induces moderate IL-6 and IL-8 responses and 229E triggers a comparatively weaker IL-6 response. Exposure to Cd significantly amplified IL-6 and IL-8 production, suggesting a pro-inflammatory role, whereas Pb suppressed IL-8 expression, highlighting its potential immunomodulatory effect. The observed interactions between viral infections and metal exposure suggest that environmental toxins can modulate host inflammatory responses in a virus-specific manner. While U87 glioblastoma cells serve as a well-characterized model for neuroinflammatory studies, their relevance to primary neural or respiratory cells is limited. Future studies should incorporate primary astrocytes, microglia, or in vivo models to validate these findings and improve physiological relevance. Additionally, extending exposure durations will be crucial to understanding the long-term consequences of viral infections in metal-contaminated environments. Investigating other environmental pollutants, including particulate matter and additional heavy metals, will provide a broader perspective on co-exposure effects. Further mechanistic studies are warranted to elucidate the specific signaling pathways, such as the NF-κB pathway and inflammasome activation, that mediate these interactions. These insights will improve our understanding of host-pathogen interactions in environmentally relevant contexts and inform public health policies addressing combined toxicant and viral exposure.

Acknowledgment

We thank the publisher for their support of the publication of this research article. We appreciate the efforts of the editorial team in reviewing and editing our work and we are thankful for the opportunity to contribute to the field of research through this publication.

Funding Information

This research received no external funding.

Author's Contributions

Hyeokjun Yun: Performed software and formal analyses. Validated and conducted investigations. Drafted the manuscript. Visualized the data.

Qian-Wen Wang: Developed the methodology. Validated and conducted investigations. Provided resources.

Jae Kyung Kim: Conceived the research. Developed the methodology. Provided resources, reviewed the manuscript, supervised the study, and secured funding.

All authors approved the final manuscript.

Ethics

The study was conducted in accordance with the declaration of Helsinki and approved by the institutional review board of the clinical research review committee of Dankook University (institutional review board DKU, certificate No. 2022-08-020).

Conflict of Interest

The authors report there are no competing interests to declare.

Data Availability Statement

The raw data supporting the conclusions of this article will be made available by the authors on request.

References

Abdelaziz, O. S., & Waffa, Z. (2020). Neuropathogenic human coronaviruses: A review. *Reviews in Medical Virology*, *30*(5). https://doi.org/10.1002/rmv.2118

Al Kahtani, M. (2020). Effect of both selenium and biosynthesized nanoselenium particles on cadmium-induced neurotoxicity in albino rats. *Human & Experimental Toxicology*, *39*(2), 159-172. https://doi.org/10.1177/0960327119880589

Alam, S. B., Willows, S., Kulka, M., & Sandhu, J. K. (2020). Severe acute respiratory syndrome coronavirus 2 may be an underappreciated pathogen of the central nervous system. *European Journal of Neurology*, *27*(11), 2348-2360. https://doi.org/10.1111/ene.14442

Bohmwald, K., Gálvez, N. M. S., Canedo-Marroquín, G., Pizarro-Ortega, M. S., Andrade-Parra, C., Gómez-Santander, F., & Kalergis, A. M. (2019). Contribution of Cytokines to Tissue Damage During Human Respiratory Syncytial Virus Infection. *Frontiers in Immunology*, *10*. https://doi.org/10.3389/fimmu.2019.00452

Boskabady, M., Marefati, N., Farkhondeh, T., Shakeri, F., Farshbaf, A., & Boskabady, M. H. (2018). The effect of environmental lead exposure on human health and the contribution of inflammatory mechanisms, a review. *Environment International*, *120*, 404-420. https://doi.org/10.1016/j.envint.2018.08.013

Budden, K. F., Shukla, S. D., Rehman, S. F., Bowerman, K. L., Keely, S., Hugenholtz, P., Armstrong-James, D. P. H., Adcock, I. M., Chotirmall, S. H., Chung, K. F., & Hansbro, P. M. (2019). Functional effects of the microbiota in chronic respiratory disease. *The Lancet Respiratory Medicine*, *7*(10), 907-920. https://doi.org/10.1016/s2213-2600(18)30510-1

Chen, P., Miah, M. R., & Aschner, M. (2016). Metals and neurodegeneration. *FASEB Journal*, *30*(11), 3666-3681.

Cheng, H., Yang, B., Ke, T., Li, S., Yang, X., Aschner, M., & Chen, P. (2021). Mechanisms of Metal-Induced Mitochondrial Dysfunction in Neurological Disorders. *Toxics*, *9*(6), 142. https://doi.org/10.3390/toxics9060142

Clark, M. J., Homer, N., O'Connor, B. D., Chen, Z., Eskin, A., Lee, H., Merriman, B., & Nelson, S. F. (2010). U87MG Decoded: The Genomic Sequence of a Cytogenetically Aberrant Human Cancer Cell Line. *PLoS Genetics*, *6*(1), e1000832. https://doi.org/10.1371/journal.pgen.1000832

Correia, A. O., Feitosa, P. W. G., Moreira, J. L. de S., Nogueira, S. Á. R., Fonseca, R. B., & Nobre, M. E. P. (2020). Neurological manifestations of COVID-19 and other coronaviruses: A systematic review. *Neurology, Psychiatry and Brain Research*, *37*, 27-32. https://doi.org/10.1016/j.npbr.2020.05.008

Desai, I., Manchanda, R., Kumar, N., Tiwari, A., & Kumar, M. (2021). Neurological manifestations of coronavirus disease 2019: exploring past to understand present. In *Neurological Sciences* (Vol. 42, Issue 3, pp. 773-785). https://doi.org/10.1007/s10072-020-04964-8

Ganguly, K., Levänen, B., Palmberg, L., Åkesson, A., & Lindén, A. (2018). Cadmium in tobacco smokers: a neglected link to lung disease? *European Respiratory Review*, *27*(147), 170122. https://doi.org/10.1183/16000617.0122-2017

Ge, Y., Song, X., Chen, L., Hu, D., Hua, L., Cui, Y., Liu, J., An, Z., Yin, Z., & Ning, H. (2019). Cadmium induces actin cytoskeleton alterations and dysfunction in Neuro-2a cells. *Environmental Toxicology*, *34*(4), 469-475. https://doi.org/10.1002/tox.22700

Grebenciucova, E., & Flanagan, E. P. (2020). IL-6: A key cytokine in neurologic diseases. *Neurology Neuroimmunology & Neuroinflammation*, *7*(5), 667.

He, W., Li, Y., Tian, J., Jiang, N., Du, B., & Peng, Y. (2015). Optimized mixture of AS, CD and PB induce mitochondria-mediated apoptosis in C6-glioma via astroglial activation, inflammation and P38-MAPK. *American Journal of Cancer Research*, *5*(8), 2396-2408.

Jakhmola, S., & Jha, H. C. (2021). Glial cell response to Epstein-Barr Virus infection: A plausible contribution to virus-associated inflammatory reactions in the brain. *Virology*, *559*, 182-195. https://doi.org/10.1016/j.virol.2021.04.005

Jarrell, Z. R., Lee, C.-M., Kim, K.-H., He, X., Smith, M. R., Raha, J. R., Bhatnagar, N., Orr, M., Kang, S.-M., Chen, Y., Jones, D. P., & Go, Y.-M. (2024). Metabolic reprograming and increased inflammation by cadmium exposure following early-life respiratory syncytial virus infection: the involvement of protein S-palmitoylation. *Toxicological Sciences*, *197*(2), 186-196. https://doi.org/10.1093/toxsci/kfad112

Jeon, J.-S., Oh, E. J., Wang, Q.-W., & Kim, J. K. (2023). Early Responses of Neural Cells (U87) to Particulate Matter Exposure: Cell Viability and Cytokine Concentration. *American Journal of Biochemistry and Biotechnology*, *19*(1), 65-70. https://doi.org/10.3844/ajbbsp.2023.65.70

Lang, R., Li, H., Luo, X., Liu, C., Zhang, Y., Guo, S., Xu, J., Bao, C., Dong, W., & Yu, Y. (2022). Expression and mechanisms of interferon-stimulated genes in viral infection of the central nervous system (CNS) and neurological diseases. *Frontiers in Immunology*, *13*. https://doi.org/10.3389/fimmu.2022.1008072

Lee, A. J., & Ashkar, A. A. (2018). The Dual Nature of Type I and Type II Interferons. *Frontiers in Immunology*, *9*. https://doi.org/10.3389/fimmu.2018.02061

Li, Y., Li, H., Fan, R., Wen, B., Zhang, J., Cao, X., Wang, C., Song, Z., Li, S., Li, X., Lv, X., Qu, X., Huang, R., & Liu, W. (2016). Coronavirus Infections in the Central Nervous System and Respiratory Tract Show Distinct Features in Hospitalized Children. *Intervirology*, *59*(3), 163-169. https://doi.org/10.1159/000453066

Liu, X., Ouyang, W., Shu, Y., Tian, Y., Feng, Y., Zhang, T., & Chen, W. (2019). Incorporating bioaccessibility into health risk assessment of heavy metals in particulate matter originated from different sources of atmospheric pollution. *Environmental Pollution*, *254*, 113113. https://doi.org/10.1016/j.envpol.2019.113113

Oh, E. J., Jeon, J.-S., Wang, Q.-W., & Kim, J. K. (2023a). Early immune response of neuronal cells (U87) to heavy metal Cd or Pb exposure. *Environmental Analysis Health and Toxicology*, *38*(1), e2023004. https://doi.org/10.5620/eaht.2023004

Oh, E. J., Jeon, J.-S., Wang, Q.-W., & Kim, J. K. (2023b). Responses of Neural Cells (U87) to HCoV-229E and HCoV-OC43 Infections. *American Journal of Biochemistry and Biotechnology*, *19*(2), 169-174. https://doi.org/10.3844/ajbbsp.2023.169.174

Pervolaraki, K., Rastgou Talemi, S., Albrecht, D., Bormann, F., Bamford, C., Mendoza, J. L., Garcia, K. C., McLauchlan, J., Höfer, T., Stanifer, M. L., & Boulant, S. (2018). Differential induction of interferon stimulated genes between type I and type III interferons is independent of interferon receptor abundance. *PLOS Pathogens*, *14*(11), e1007420. https://doi.org/10.1371/journal.ppat.1007420

Ramachandran, R., Jayaraman, A., & Srinivasan, P. (2021). Heavy metals and immune modulation in viral pathogenesis: the role of cadmium and lead in immune signaling. *Toxicology Letters*, *347*, 1-9. https://doi.org/10.1016/j.toxlet.2021.08.003

Vilotić, A., Nacka-Aleksić, M., Pirković, A., Bojić-Trbojević, Ž., Dekanski, D., & Jovanović Krivokuća, M. (2022). IL-6 and IL-8: An Overview of Their Roles in Healthy and Pathological Pregnancies. *International Journal of Molecular Sciences*, *23*(23), 14574. https://doi.org/10.3390/ijms232314574

Wang, Q., Jeon, J., & Kim, J. K. (2022). Effects of PM10 exposure at low concentrations on the cell viability and cytotoxicity of A549. *China Tropical Medicine*, *22*, 315-319. https://doi.org/10.13604/j.cnki.46-1064/r.2022.04.05

Wang, Q. W., Jeon, J. S., & Kim, J. K. (2022). Effects of heavy metals on the viability of A549 cells. *Indian Journal of Biochemistry and Biophysics*, *59*, 726-731.

Wang, Q. W., & Kim, J. K. (2024). Cytokine Expression During the Early Response of A549 Cells to Infection with Influenza Virus, *Alphacoronavirus* and *Betacoronavirus*. *American Journal of Biochemistry and Biotechnology*, *20*(2), 151-158. https://doi.org/10.3844/ajbbsp.2024.151.158

Wu, L., Lu, X., Zhang, S., Zhong, Y., Gao, H., Tao, F., & Wu, X. (2024). Co-exposure effects of urinary polycyclic aromatic hydrocarbons and metals on lung function: mediating role of systematic inflammation. *BMC Pulmonary Medicine*, *24*(1). https://doi.org/10.1186/s12890-024-03173-9

Wu, X., Cobbina, S. J., Mao, G., Xu, H., Zhang, Z., & Yang, L. (2016). A Review of Toxicity and Mechanisms of Individual and Mixtures of Heavy Metals in the Environment. *Environmental Science and Pollution Research*, *23*(9), 8244-8259. https://doi.org/10.1007/s11356-016-6333-x

Xu, G., Xia, Z., & Deng, F. (2023). UV-inactivated viruses and their roles in immune activation. *Journal of Virology*, *97*(4), 00144-23.

Yin, X., Xia, K., Peng, S., Tan, B., Huang, Y., Wang, M., & He, M. (2024). ABCF1/CXCL12/CXCR4 Enhances Glioblastoma Cell Proliferation, Migration, and Invasion by Activating the PI3K/AKT Signal Pathway. *Developmental Neuroscience*, *46*(3), 210-220. https://doi.org/10.1159/000533130

Yin, Y., & Wunderink, R. G. (2018). MERS, SARS and other coronaviruses as causes of pneumonia. *Respirology*, *23*(2), 130-137. https://doi.org/10.1111/resp.13196

Yuan, Y., Wu, Y., Ge, X., Nie, D., Wang, M., Zhou, H., & Chen, M. (2019). In vitro toxicity evaluation of heavy metals in urban air particulate matter on human lung epithelial cells. *Science of The Total Environment*, *678*, 301-308. https://doi.org/10.1016/j.scitotenv.2019.04.431

Zhang, H., Wang, J., Zhang, K., Shi, J., Gao, Y., Zheng, J., He, J., Zhang, J., Song, Y., Zhang, R., Shi, X., Jin, L., & Li, H. (2024). Association between heavy metals exposure and persistent infections: the mediating role of immune function. *Frontiers in Public Health*, *12*. https://doi.org/10.3389/fpubh.2024.1367644

Zhang, H., Yan, J., Niu, J., Wang, H., & Li, X. (2022). Association between lead and cadmium co-exposure and systemic immune inflammation in residents living near a mining and smelting area in NW China. *Chemosphere*, *287*, 132190. https://doi.org/10.1016/j.chemosphere.2021.132190