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ORIGINAL PAPER

Cytotoxicity induced by the mixture components of nickel and poly aromatic hydrocarbons

Injeong Kim · Yeseul Lee · Sang Don Kim

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Abstract Although particulate matter (PM) is composed of various chemicals, investigations regarding the toxicity that results from mixing the substances in PM are insufficient. In this study, the effects of low levels of three PAHs (benz[a]anthracene, benzo[a]-pyrene, and dibenz[a,h]anthracene) on Ni toxicity were investigated to assess the combined effect of Ni–PAHs on the environment. We compared the difference in cell mortality and total glutathione (tGSH) reduction between single Ni and Ni–PAHs co-exposure using A549 (human alveolar carcinoma). In addition, we measured the change in Ni solubility in chloroform that was triggered by PAHs to confirm the existence of cation–π interactions between Ni and PAHs. In the single Ni exposure, the dose–response curve of cell mortality and tGSH reduction were very similar, indicating that cell death was mediated by the oxidative stress. However, 10 μM PAHs induced a depleted tGSH reduction compared to single Ni without a change in cell mortality. The solubility of Ni in chloroform was greatly enhanced by the addition of benz[a]anthracene, which demonstrates the cation–π interactions between Ni and PAHs. Ni–PAH complexes can change the toxicity mechanisms of Ni from oxidative stress to others due to the reduction of Ni^{2+} bioavailability and the accumulation of Ni–PAH complexes on cell membranes. The abundant PAHs contained in PM have strong potential to interact with metals, which can affect the toxicity of the metal. Therefore, the mixture toxicity and interactions between diverse metals and PAHs in PM should be investigated in the future.

Keywords Mixture toxicity · Oxidative stress · Total glutathione · Cation–π interaction · π donor

I. Kim · Y. Lee · S. D. Kim (✉)
School of Earth Sciences and Environmental Engineering, Gwangju Institute of Science and Technology (GIST), 123 Cheomdangwagi-ro, Buk-gu, Gwangju 61005, South Korea
e-mail: sdkim@gist.ac.kr

Introduction

Particulate matter (PM), a mixture of solid or liquid matter found in the air, has gained considerable attention as a major air pollutant in recent years. The small size of PM, such as PM2.5 (aerodynamic size < 2.5 μm), can reach alveoli and cause lung diseases such as coughs, asthma, and lung cancer (POPE III 2000). The annual average PM2.5 concentration from 2006 to 2011 in Seoul (the capital of South Korea) was 27 $\mu g/m^3$, which is higher than the annual mean established by the WHO (10 $\mu g/m^3$) (Lee 2014) and indicates a high level of PM exposure. The prime criteria for preventing the adverse effects of PM are based on the mass concentration, but recent research has shown that the chemical composition of PM can be a more important factor for determining

PM toxicity than mass concentration (Yang et al. 2016; Dergham et al. 2015; Steenhof et al. 2011).

PM is composed of diverse chemicals such as inorganic sulfates and nitrates, black carbon, metal, organic compounds, and others. The adverse health effects of inorganic sulfates and nitrates are insignificant despite high concentrations in PM. However, metal and organic compounds, especially polycyclic aromatic hydrocarbons (PAHs), are considered to play an important role in PM toxicity (WHO and UNAIDS 2006). The primary toxic effect of metals is that they generate oxidative stress that is mediated by reactive oxygen species (ROS) production (Kim et al. 2015), whereas PAHs cause genotoxicity and carcinogenicity through the metabolic activation of PAHs (Valavanidis et al. 2008). However, the exposure to metals and PAHs occurs concurrently because they both compose PM, and the toxicity mechanisms of individual metals and PAHs work differently in the human body but can affect each other biologically and chemically.

Several studies have reported the interactions between metal and PAHs and their effect on the toxicity to aquatic organisms (Gust and Fleeger 2006; Gauthier et al. 2015), microalgae (Wang et al. 2013), bacteria (Xiao et al. 2007), and human cells (Muthusamy et al. 2016a, b; Honda et al. 2017). The metal–PAH mixtures showed the synergism, additivity, and antagonism effects depending on the chemicals and organisms tested (Gauthier et al. 2014). This research revealed that the co-exposure of metal–PAHs has an influence on the uptake of chemicals (Xiao et al. 2007; Gust and Fleeger 2006), antioxidant responses (Wang et al. 2013; Muthusamy et al. 2016b; Gauthier et al. 2015) and the metabolic activation of PAHs (Benedetti et al. 2007). The combined effects of metal–PAHs were induced not only by the biological responses to the toxicity mechanisms of metals and PAHs but also by the chemical interactions between metal–PAHs. A cation–π interaction includes noncovalent bonding between a cationic compound (i.e., metal) and an electron–rich π system is formed by aromatic compounds (Ma and Dougherty 1997). It has been reported that cation–π interactions can cause fluctuations in chemical bioavailability (Xiao et al. 2007; Tao et al. 2015; Gauthier et al. 2014). Therefore, investigations of the toxicity of metal–PAH mixtures should consider both the changes in biological effects and the chemical interactions between metals and PAHs.

Nickel and heavy PAHs are the important chemicals in PM. Ni is a naturally abundant element and it is widely used in industrial activities such as electroplating, welling, refining, and alloy production. Ni is a carcinogenic compound and it has been established that Ni in PM is associated with an increase in respiratory and cardiovascular disease and morbidity (Zhang et al. 2009; Lippmann et al. 2006). The bioaccessiblity of Ni in PM is approximately 30–100%, which is much higher than other metals (Voutsa and Samara 2002). Reported Ni concentrations in PM range from tens to hundreds of ng/m^3 (Kasprzak et al. 2003). PAHs are primarily generated by the incomplete combustion of coal, oil, and wood. Some PAHs are classified as carcinogens and correlations between lung cancer and PAHs have been reported (Moorthy et al. 2015). The characteristics of PAHs vary depending on the PAH species. Generally, PAHs with a heavier molecular weight (e.g., benzo[a]pyrene, benz[a]anthracene and dibenz[a,h]anthracene) are more easily sorbed to particles than light PAHs due to low vapor pressure (Abdel-Shafy and Mansour 2016). The reported concentrations of individual PAHs are lower than Ni, ranging from a few to tens of ng/m^3 (Abdel-Shafy and Mansour 2016; Ravindra et al. 2001).

In this study, Ni and three PAHs (dibenz[a,h]anthracene, benzo[a]pyrene, and benz[a]anthracene) were selected as the major toxicants in PM. To assess the combined effect of Ni–PAHs, the mixed ratio between Ni and PAHs is important to constrain because their combined effect can differ based on the mixture ratio (Jensen and Krøkje 2008). Considering the environmental concentration range, the effect of no effect level PAHs (10 μM) to Ni toxicity was evaluated. The cytotoxicity and oxidative stress were measured after the exposure to Ni and an Ni–PAHs mixture using A549 (human alveolar carcinoma cell). The chemical interactions (especially the cation–π interaction) of Ni–PAHs were also confirmed in the chemical experiment. Finally, we discuss the possible toxicity mechanisms induced by the co-exposure of Ni–PAHs based on the observed biological and chemical results and the indicators that can be used to predict the occurrence of cation–π interactions between metals and PAHs.

Materials and methods

Materials

An Ni(II) aqueous stock solution was prepared by dissolving nickel chloride hexahydrate ($NiCl_2 \cdot 6H_2O$, $\geq$ 96%) (Showa Chemical, Japan) in deionized water (DW). Benz[a]anthracene (BaA, $\geq$ 99%), benzo[a]-pyrene (BaP, $\geq$ 96%) (Sigma-Aldrich, USA) and dibenz[a,h]anthracene (DBahA, $\geq$ 99%) (Cerilliant, USA) were diluted with dimethyl sulfoxide (DMSO, $\geq$ 95%) (Sigma-Aldrich, USA). Prior to exposure to cells, the pre-diluted Ni solution of the desired concentration was mixed with fresh cell culture media in the ratio of 1:9. PAH stock solutions were also mixed with fresh cell culture media by maintaining the final concentration of DMSO within 1% (v/v) just before conducting the bioassay.

Cytotoxicity

Cell culture

The A549 (human alveolar carcinoma) cell line was obtained from the Korea Cell Line Bank and was used in all cytotoxicity experiments. The cells were cultured in the Roswell Park Memorial Institute medium (RPMI) (Lonza, Switzerland) with 5% fetal bovine serum (GIBCO, USA) and 1% penicillin–streptomycin (GIBCO, USA) solutions to prevent bacterial contamination of the cell cultures. Cell culture media were changed every other day. A subculture of cells was conducted every 2 or 3 days after reaching 80% confluence. The cells were incubated in a humidified CO_2 incubator at 37 °C in 5% CO_2.

For the cytotoxicity experiment, the A549 cells were seeded in 96-well cell culture plates and 6-well cell culture plates at 4×10^4 and 4×10^5 cells/well, respectively. The passage numbers of cells used in all bioassays were below 20 to obtain consistent results. After 24 h, the cell culture media were carefully removed and the chemicals mixed with cell culture media were gently exposed to A549. Exposed cells were kept in the CO_2 incubator at 37 °C in 5% CO_2 for 24 h.

Cell mortality

Cell death was measured using a WST-1 assay. Red-colored, water-soluble tetrazolium salt in Premix WST-1 solution (Takara Bio Inc., Japan) can change to orange-colored after exposure to mitochondrial dehydrogenase, which is only active in living cells. After 24 h of exposure, the cells in the 96-well cell culture plates were washed in each well using Dulbecco's Phosphate-Buffered Saline (DPBS) (Lonza, Switzerland), and the diluted WST-1 solution with added RPMI (with a ratio of one to ten) was added to each well under shaded conditions. After the 2 h period of post-incubation in a CO_2 incubator, the absorbance (ABS) was measured at 440 and 690 nm using a microplate spectrophotometer (Bio-Tek, USA). Cell death was calculated using the following equation:

$$\text{Cell death}\,(\%) = \left(1 - \frac{\text{ABS}_{\text{experiment cells}} - \text{ABS}_{\text{background}}}{\text{ABS}_{\text{control cells}} - \text{ABS}_{\text{background}}}\right).$$

Oxidative stress level

The decreased level of intracellular total glutathione (tGSH) represents the oxidative stress level within the cell. tGSH was analyzed using a Glutathione Assay Kit (Sigma-Aldrich, USA). tGSH was determined by measuring the total amount of glutathione (GSH) and glutathione disulfide (GSSG). The treated A549 cells that had been incubated in the 6-well cell culture plates were trypsinized and pelleted by centrifugation at $600 \times g$ for 5 min. The cell pellets were resuspended in an adequate amount of 5% 5-sulfosalicilic acid. Freezing and thawing of the cells were done using a water bath and liquid nitrogen up to 3 times. After centrifugation at $10{,}000 \times g$ for 10 min, the supernatants were used for the quantification of tGSH, and the cell pellets were used for the quantification of proteins. The supernatants and GSH standard solution were added to the well plate. Then, a mixture of glutathione reductase (GR) and 5,5′-dithiobis (2-nitrobenzoic acid) was injected into the supernatants and GSH standard solution. After incubation at room temperature for 5 min, 50 μL of nicotinamide adenine dinucleotide phosphate solution was added to start the GR reaction. The concentration of tGSH was determined by kinetic measurement for 5 min using microplate spectrophotometer at 412 nm. The decreased level of tGSH was calculated using the method that was used for measuring cell death.

Protein quantification

For normalizing tGSH concentrations to protein concentration, the amount of protein was quantified using a Bradford assay that used a Protein Assay Dye Reagent Concentrate (Bio-Rad, USA). Cell pellets and bovine serum albumin (a protein standard) were dissolved in 0.5 N NaOH solutions. Then, 10 µL samples were added into each well of the 96-well plate, followed by the addition of 200 µL diluted dye reagent solution. After maintaining the mixture at room temperature in the dark for 5 min, the absorbance at 595 nm was measured using a microplate spectrophotometer.

Saturation solubility of nickel

To confirm the chemical interactions between Ni and PAHs, the saturation solubility of Ni in chloroform ($\geq$ 99.8%, Sigma-Aldrich, USA) was analyzed. The saturation solubility experiment was conducted following the methods of Qu et al. (2007) with slight modification. 1,2,4-Trichlorobenzene (1,2,4-TCB, $\geq$ 99%) (Sigma-Aldrich, USA) and naphthalene (NAPH, 99%) (Sigma-Aldrich, USA) were used as negative controls. One hundred ppb of Ni was dissolved in DW and aromatic compounds were diluted to the desired concentration with chloroform. Fifteen milliliters of both the Ni and aromatic compound solutions were mixed into a 40-mL amber glass vial. After vortexing for 5 min, the mixtures of Ni and aromatic compounds were shaken using a shaking incubator for 3 h at 170 rpm and 25 °C. Based on preliminary experiments, 3 h was a sufficient amount of time to reach equilibrium. The Ni and aromatic compound solutions were allowed to separate for 1 h. Then, 10 mL of chloroform was extracted from the amber vial using a syringe and was transferred into a new amber vial that contained 10 mL of 2% HNO_3 (70% HNO_3 was diluted using DW, Sigma-Aldrich, USA) as a stripping solution. Afterward, the extracted chloroform in the new vials was vortexed for 5 min, followed by shaking for 3 h at 25 °C and 170 rpm to reach equilibrium. Then, the mixture of extracted chloroform and 2% HNO_3 was left to settle on the flat desk for 1 h to allow the separation of the 2% HNO_3 from the chloroform. Seven-milliliter supernatants of 2% HNO_3 were sampled using a syringe. The concentration of Ni^{2+} in 2% HNO_3 was analyzed using an inductively coupled plasma mass spectrometer (Agilent 7500ce, Agilent, USA).

Results and discussion

Cytotoxicity induced by a single compound

The results confirmed that cell death and oxidative stress were induced by exposure to Ni using A549 (Fig. 1). The dose–response curve of cell mortality and total glutathione (tGSH) reduction shows Ni concentration-dependent toxicity and great similarity. The reduction of tGSH indicates severe oxidative stress in the cell. GSH is an important protective antioxidant that maintains redox condition in the cell. tGSH is the sum of GSH and GSSG, which is an oxidized form of GSH. When reactive oxygen species (ROS) are produced within the cell, GSH directly reacts with ROS and oxidizes as GSSG. GSSG can be reduced to GSH by nicotinamide adenine dinucleotide phosphate (NADPH), which is an important antioxidant mechanism in the cell. However, excessive ROS leads to the accumulation of GSSG within the cell. To maintain a redox equilibrium in the cell, GSSG is released from the cell and tGSH is reduced (Lu 2009). Therefore, the dose-dependent depletion of tGSH indicates that a significant amount of ROS was produced by Ni exposure to the cell.

The main transport routes of soluble Ni (Ni^{2+}) to the cell are through the cell membrane by passive diffusion and ion channels, such as the Ca^{2+} or Fe^{2+}

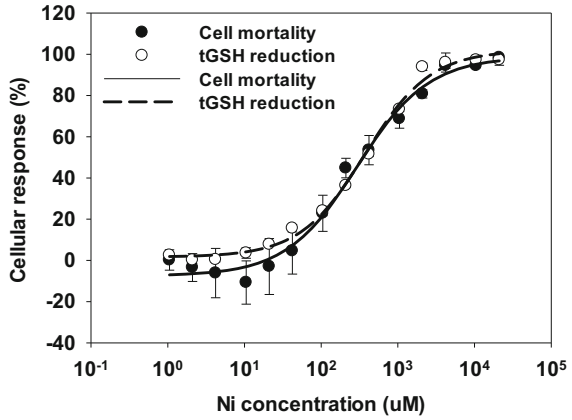

Fig. 1 Cell mortality and total glutathione (tGSH) reduction of A549 by Ni exposure. Line indicates the dose–response curve for each cellular response

channel. Ni produces ROS in the cell through Fenton-type reactions (Lee et al. 2012; Kasprzak et al. 2003). Ni-generated ROS has been demonstrated to be associated with DNA damage, carcinogenesis, apoptosis and other diseases (Das et al. 2008; Pulido and Parrish 2003). Significant similarity was observed between the dose–response curves of tGSH and cell mortality. This result suggests that there is a strong relationship between oxidative stress and cell death induced by Ni^{2+}.

For benz[a]anthracene (BaA), benzo[a]pyrene (BaP) and dibenz[a,h]anthracene (DBahA), there were no differences in cell mortality and tGSH at 1, 10, and 100 μM for all PAHs (data not shown). One hundred micromole is a maximum exposure concentration of PAHs due to their low solubility in DMSO and low mixing ratio of DMSO in cell culture media (1% v/v). Based on these results, 10 μM PAHs was selected as the no effect level to observe toxicity changes of Ni. The toxicity mechanism of the PAHs is totally different from the toxicity mechanism of Ni. Carcinogenesis is a major toxic effect of PAHs. PAHs, as lipophilic compounds, can pass through the cell membrane by passive diffusion (Moorthy et al. 2015). However, parent PAH compounds are commonly considered as biologically inactive. Metabolites that are produced by the metabolism of PAHs (especially cytochrome P4501A (CYP1A) activation) make the PAHs toxic. PAH metabolites and DNA adducts that are induced by their reaction with DNA play a considerable role in PAH-related carcinogenesis (Moorthy et al. 2015; Gauthier et al. 2014). Since the cytotoxicity of PAHs was not observed in this study, we interpret that 1, 10 and 100 μM of PAHs do not induce cytotoxicity on A549.

Combined effects on cellular response

During exposure to only Ni, there was a similarity in cellular response regarding mortality and tGSH reduction. Although exposure concentration of PAHs was at the no effect level, significant differences between mortality and tGSH reduction were induced by the co-exposure to Ni–PAHs (Fig. 2). tGSH reduction decreased slightly compared to cell mortality for all PAHs. Especially in Ni + BaA, considerable cell death occurred without a noticeable change in tGSH reduction. These results indicate that the no effect level of PAHs can change Ni toxicity mechanisms and cell death can be affected by toxicity mechanisms that are not associated with oxidative stress.

Figure 3 shows the difference between the cellular response to Ni alone and to Ni–PAHs mixtures. Cell mortality was not affected by co-exposure, but tGSH reduction of the Ni–PAHs mixture was depleted after co-exposure compared to Ni alone. To gain a better understanding of the effect of PAHs on Ni toxicity, we calculated and compared the IC_{10} (inhibitory concentration of 10%) of each response. IC_{10} is a suitable indicator of the effect of PAHs on Ni toxicity because it is not only more realistic than no observed effect concentration (NOEC) (Perina et al. 2011), but it is also calculated from in vitro tests and is correlated with a median lethal dose (LD_{50}) that is derived from in vivo tests (Jover et al. 1994). Using the IC_{10} comparison, a small perturbation in cell mortality was detected between Ni and Ni–PAHs (Table 1). In tGSH reduction, there was a marginal negative effect. The IC_{10} of tGSH reduction increased significantly, approximately 3–6 times, when Ni and PAHs coexisted.

Much research on the mixture toxicity of metal and PAHs has reported a metal-induced inhibition of CYP1A enzymes (Benedetti et al. 2007, 2009; Gauthier et al. 2014; Jensen and Krøkje 2008). However, the inhibition of CYP1A is insufficient to interpret the results of this study. CYP1A metabolizes PAH compounds through phase I reactions in the PAH detoxification pathway and produces genotoxic PAH metabolites. The down-regulation of CYP1A by metal leads to a decrease in PAHs metabolites, and the genotoxic effect is mediated by PAHs metabolites (e.g., antagonistic effect). Although it has been reported that the inhibition of CYP1A accompanies oxidative perturbations, such as an increase of heme oxygenase (HO-1) and the fluctuation of other antioxidant enzymes (Gauthier et al. 2014; Benedetti et al. 2007, 2009; Muthusamy et al. 2016a), there is a considerable difference in the exposure concentration of the mixtures used in previous studies and this study. Furthermore, previous studies did not investigate the amount of change of tGSH between Ni alone and the Ni–PAHs mixture (Benedetti et al. 2007, 2009). Given the Ni toxicity mechanism, the difference between the cellular response of Ni and Ni–PAHs can be caused by the fluctuation of Ni uptake or ROS production.

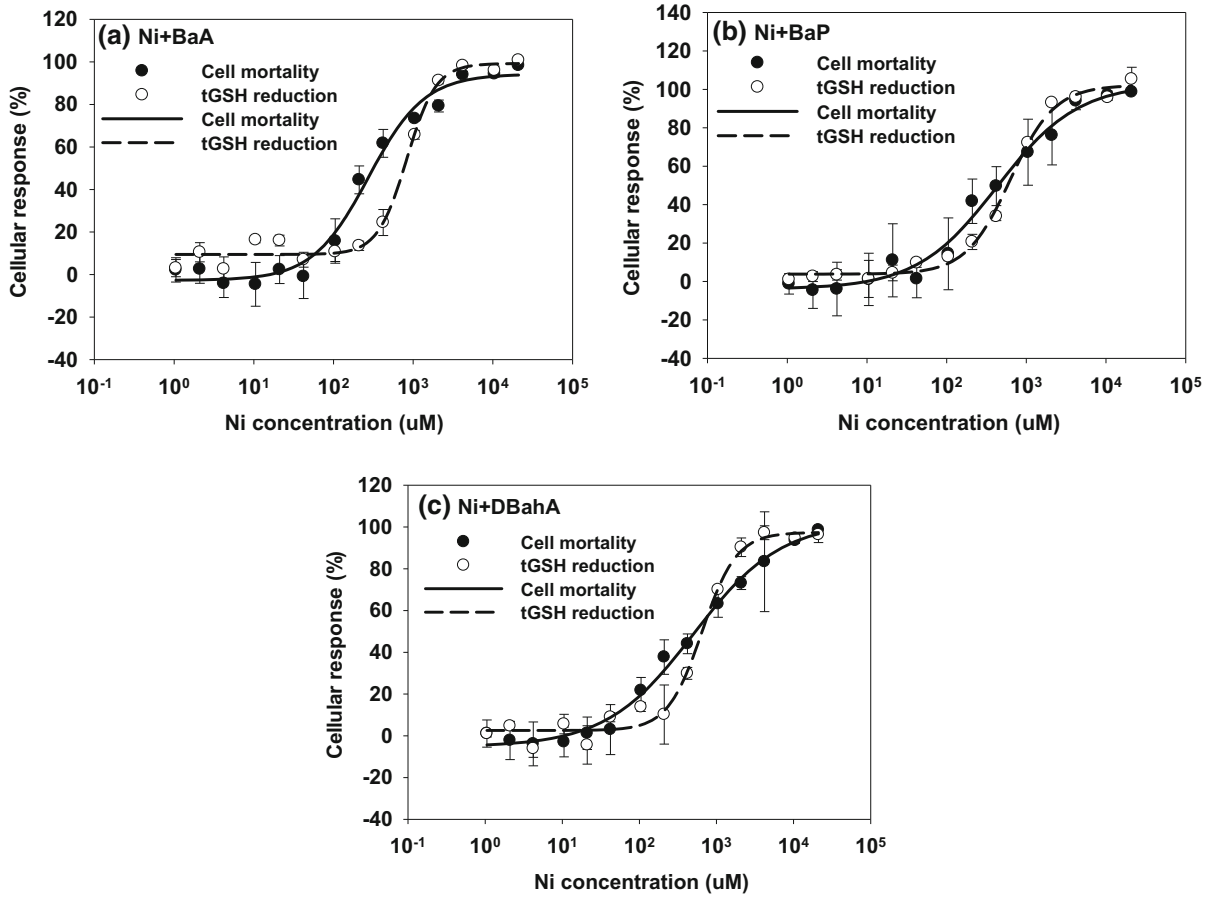

Fig. 2 Cell mortality and total glutathione (tGSH) reduction of A549 by Ni exposure with **a** BaA, **b** BaP and **c** DBahA. Line indicates the dose–response curve for each cellular response

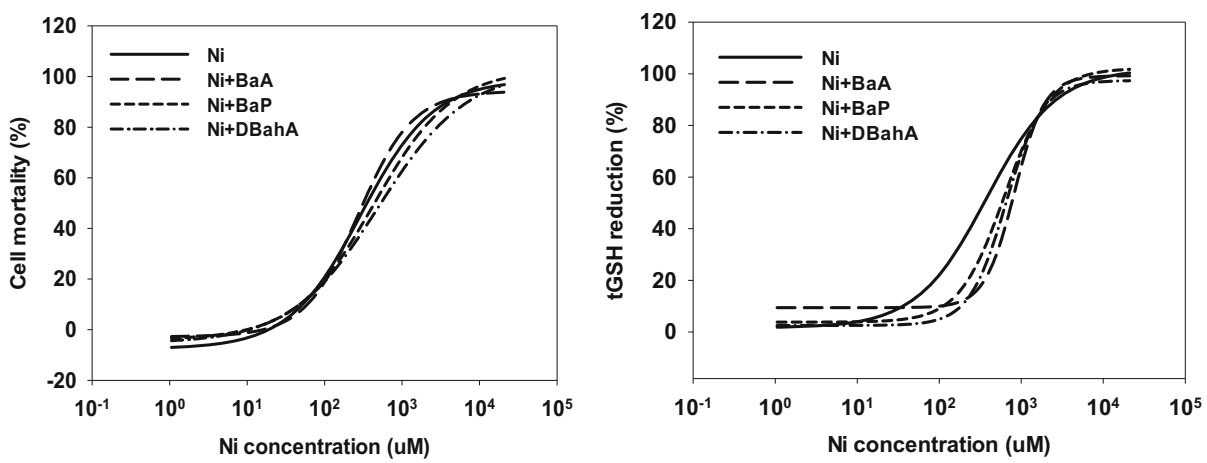

Fig. 3 Dose–response curve for Ni and Ni–PAHs mixture for cell mortality (left) and total glutathione (tGSH) reduction (right)

Table 1 IC_{10} (10% inhibitory concentration) of cell mortality and total glutathione (tGSH) reduction between Ni and Ni–PAHs mixture to A549 cell

	Ni	Ni + BaA	Ni + BaP	Ni + DBahA
IC_{10} for cell mortality (μM)	52.14	59.89	47.68	47.80
IC_{10} for tGSH reduction (μM)	36.13	98.39	111.81	184.06

Chemical reaction between Ni and PAHs

Chemical interaction is one of the factors that influences the mixture toxicity by affecting the uptake rate or free ion concentration of the toxicant (Tao et al. 2015). Since the exposure concentration of PAHs was at no effect level, the toxicity of Ni should not change through the addition of PAHs if there is no interaction between the Ni and PAHs. Thus, the change in toxicity of Ni indicates that there were interactions between Ni and the PAHs. The complexation of metal and PAHs can be induced by cation–π interactions, which can affect the mixture toxicity (Xiao et al. 2007; Tao et al. 2015; Gauthier et al. 2014).

Figure 4 indirectly shows Ni–PAH complexation. Ni is rarely dissolved in chloroform. However, Ni–PAH complexation can enhance the solubility of Ni in chloroform. BaA improved the Ni solubility in chloroform up to 72 times in 40 μM of chloroform. However, the solubility of Ni in chloroform was not affected by naphthalene (NAPH) or 1,2,4-trichlorobenzene (1,2,4-TCB). NAPH is a week π-donor and 1,2,4-TCB is a non-π-donor. No change in the solubility of Ni by the concentration of NAPH and 1,2,4-TCB indicates that NAPH and 1,2,4-TCB do not induce cation–π interactions. Unlike NAPH and 1,2,4-TCB, the significant increase in solubility of Ni by BaA implies that BaA combines strongly with Ni by strong cation–π interactions.

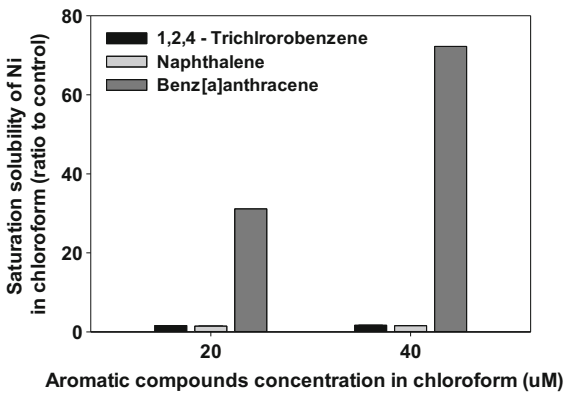

Fig. 4 Saturation solubility of Ni in chloroform by non-π-donor (1,2,4-trichlorobenzene), a weak π-donor (naphthalene) and a strong π-donor (benz[a]anthracene)

It is well known that free metal ions are more bioavailable than metals complexed with other chemicals (Gauthier et al. 2014; Van Leeuwen 2000). Ni–PAH complexation results in a decrease of free Ni^{2+} and Ni^{2+} uptake by the cell. In addition, ROS production can be reduced by inhibiting the Fenton reaction of Ni^{2+}. The reduced Ni^{2+} uptake and ROS production can cause a decrease in tGSH reduction. However, although the bioavailability of Ni^{2+} decreased, the cell mortality did not diminish. These results indicate that the Ni–PAH complex affects the cell mortality. Metal–PAH complexing promotes the accumulation of metal on the cell membrane and leads to cell membrane damage (Gauthier et al. 2014). Therefore, the observed cell death during co-exposure to Ni and PAHs resulted from both ROS production by free Ni^{2+} and cell membrane damage by Ni–PAH complex.

Cation–π interactions can contribute to Ni toxicity at no effect level of PAHs. Several studies have revealed the effects of cation–π interactions on organisms such as cyanobacteria (Tao et al. 2013, 2015) and *Escherichia Coli* (Xiao et al. 2007). Since cation–π interactions have a non-negligible influence on the biological system, the possibility of predicting cation–π interactions between metals and PAHs can provide valuable insight into understanding the effect of PAHs on metal toxicity. The main driving force behind cation–π interaction is electrostatic interactions (Vijay and Sastry 2008; Mahadevi and Sastry 2012), but other mechanisms such as charge transfer also contribute slightly to cation–π interaction (Aschi et al. 2002). Therefore, the driving force can differ depending on the nature of cationic compound and the π system. In the case of metal–PAHs, the

electrostatic interactions between metal cations and the quadruple moment of PAHs plays an important role in cation–π interaction.

The nature of PAHs can be a qualitative guide to predict cation–π interactions (Ng et al. 1998). Table 2 lists the potential indicators of PAHs for estimating the occurrence of cation–π interactions between metals and PAHs. Considering PAHs, cation–π interactions are largely controlled by the size and nature of the π system. The size of the π system of the PAHs is related to the number of double bonds, the molecular weight (M_W) and the molecular surface area (SAS). It is accepted that enhanced polarization that is associated with an increase in the π system of PAHs makes cation–π interactions stronger (Vijay and Sastry 2008; Mahadevi and Sastry 2012). The quadruple moment (Q) of PAHs also involves the cation–π interactions, but its effect is relatively weak when the PAHs have more than four rings (Ng et al. 1998). The quantum properties of PAHs (HOMO, LUMO and Δorb) are related to the charge transfer from aromatic HOMO to cation LUMO. Among the quantum properties, the aromatic HOMO shows a correlation with the amount of charge transfer (Aschi et al. 2002). Together, the increase in ring number in PAHs may be a central factor that affects the cation–π interactions between metals and PAHs by expanding the number of double bonds, the M_W and the SAS. Interestingly, all parameters are related to one another, including vapor pressure (V_P). The negative correlation between M_W and V_P suggests that heavy PAHs are sorbed on to particles more than light PAHs (Abdel-Shafy and Mansour 2016). Therefore, frequently detected PAHs in PM have a strong ability to interact with cationic metals through cation–π interactions, ultimately influencing metal toxicity.

This study revealed that a low concentration of PAHs can affect the toxicity of Ni through chemical interactions, especially through cation–π interactions. It is not a predictable consequence based on conventional knowledge regarding mixture toxicity. For instance, a general prediction model for mixture toxicity, such as concentration addition and independent action, can be applied in only higher than no effect level of the pollutants. However, we observed that the primary toxicity mechanism of Ni was changed from oxidative stress to another mechanism by adding a low concentration of PAHs. This study shows the important role of trace pollutants in the environment. The toxicity in mixture system cannot be

Table 2 Physicochemical and quantum properties for 11 polycyclic aromatic hydrocarbons (PAHs)

PAHs	No. of double bond	M_W[a]	SAS[b]	Q[c]	HOMO[b]	LUMO[b]	Δorb[b]	V_P (Pa)[d]
Dibenz[a,h]anthracene	11	278.35	466.7		− 8.26	− 0.81	7.45	3.7×10^{-8}
Benzo[a]pyrene	10	252.31	439.7		− 7.92	− 1.11	6.81	7.0×10^{-7}
Benz[a]anthracene	9	228.29	426.6	− 16.91	− 8.21	− 0.81	7.39	2.8×10^{-5}
Fluoranthene	8	202.25	388.8	− 15.08	− 8.63	− 0.93	7.70	1.2×10^{-3}
Pyrene	8	202.25	372.9	− 14.69	− 8.13	− 0.89	7.24	6.0×10^{-4}
Anthracene	7	178.23	358.4	− 13.30	− 8.12	− 0.84	7.28	1.0×10^{-3}
Phenanthrene	7	178.23	357.8	− 13.30	− 8.62	− 0.41	8.21	2.0×10^{-2}
Fluorene	6	166.22	361.4		− 8.71	− 0.22	8.49	9.0×10^{-2}
Acenaphthene	5	154.22	337.8		− 8.49	− 0.21	8.28	3.0×10^{-2}
Acenaphthylene	6	152.19	323.5		− 8.94	− 0.94	8.01	9.0×10^{-1}
Naphthalene	5	128.17	304.2	− 9.77	− 8.71	− 0.27	8.45	11

M_W, molecular weight; SAS, solvent-accessible molecular surface area; HOMO, highest occupied molecular orbital; LUMO, lowest unoccupied molecular orbital; Δorb, absolute value of the difference between HOMO and LUMO; Q, quadrupole moment; V_P, vapor pressure at 25 °C

[a]Mackay et al. (2006)

[b]Baggiani et al. (2007)

[c]Ng et al. (1998)

[d]Stogiannidis and Laane (2015)

predicted by the toxicity mechanism of a major compound, even though other pollutants have very low concentration. Thus, further studies are needed regarding the toxic effects and chemical interactions between major and trace pollutants such as metals and PAHs.

Conclusion

This study investigated the effect of no effect level PAHs on the toxicity of Ni using a human alveolar carcinoma cell A549 and chemical interactions between Ni and PAHs. The total glutathione reduction by Ni was highly decreased by PAHs, whereas there was no difference in cell mortality between Ni alone and Ni–PAHs mixtures. Chemical interactions, especially cation–π interactions, between Ni and PAHs were confirmed to reduce the bioavailability of Ni by inhibiting Ni transport through ion channels, leading to cell membrane damage through the accumulation of Ni–PAHs complexes in the cell membrane. This study shows that a no effect level of pollutants can affect the toxicity mechanisms of other chemicals through chemical interaction. Therefore, further studies are necessary to assess the toxic effects and chemical interactions of no effect level pollutants in the environment.

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References

Abdel-Shafy, H. I., & Mansour, M. S. (2016). A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. *Egyptian Journal of Petroleum, 25*(1), 107–123.

Aschi, M., Mazza, F., & Di Nola, A. (2002). Cation–π interactions between ammonium ion and aromatic rings: An energy decomposition study. *Journal of Molecular Structure: THEOCHEM, 587*(1), 177–188.

Baggiani, C., Anfossi, L., Baravalle, P., Giovannoli, C., & Giraudi, G. (2007). Molecular recognition of polycyclic aromatic hydrocarbons by pyrene-imprinted microspheres. *Analytical and Bioanalytical Chemistry, 389*(2), 413–422.

Benedetti, M., Fattorini, D., Martuccio, G., Nigro, M., & Regoli, F. (2009). Interactions between trace metals (Cu, Hg, Ni, Pb) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the antarctic fish *Trematomus bernacchii*: Oxidative effects on biotransformation pathway. *Environmental Toxicology and Chemistry, 28*(4), 818–825.

Benedetti, M., Martuccio, G., Fattorini, D., Canapa, A., Barucca, M., Nigro, M., et al. (2007). Oxidative and modulatory effects of trace metals on metabolism of polycyclic aromatic hydrocarbons in the Antarctic fish *Trematomus bernacchii*. *Aquatic Toxicology, 85*(3), 167–175.

Das, K., Das, S., & Dhundasi, S. (2008). Nickel, its adverse health effects and oxidative stress. *Indian Journal of Medical Research, 128*(4), 412.

Dergham, M., Lepers, C., Verdin, A., Cazier, F., Billet, S., Courcot, D., et al. (2015). Temporal–spatial variations of the physicochemical characteristics of air pollution Particulate Matter (PM 2.5–0.3) and toxicological effects in human bronchial epithelial cells (BEAS-2B). *Environmental Research, 137,* 256–267.

Gauthier, P. T., Norwood, W. P., Prepas, E. E., & Pyle, G. G. (2014). Metal–PAH mixtures in the aquatic environment: A review of co-toxic mechanisms leading to more-than-additive outcomes. *Aquatic Toxicology, 154,* 253–269.

Gauthier, P. T., Norwood, W. P., Prepas, E. E., & Pyle, G. G. (2015). Metal–polycyclic aromatic hydrocarbon mixture toxicity in *Hyalella azteca*. 2. Metal accumulation and oxidative stress as interactive co-toxic mechanisms. *Environmental Science and Technology, 49*(19), 11780–11788.

Gust, K. A., & Fleeger, J. W. (2006). Exposure to cadmium–phenanthrene mixtures elicits complex toxic responses in the freshwater tubificid oligochaete, *Ilyodrilus templetoni*. *Archives of Environmental Contamination and Toxicology, 51*(1), 54–60.

Honda, A., Chowdhury, P. H., Ito, S., Okano, H., Onishi, T., Kawaryu, Y., et al. (2017). Synergic effects of 9,10-phenanthrenequinone and cadmium on pro-inflammatory responses in airway epithelial cells. *Environmental Toxicology and Pharmacology, 52,* 276–279.

Jensen, M. H. S., & Krøkje, Å. (2008). Application of statistical experimental design and multivariate data analysis for evaluation of mixtures using cytochrome P4501A induction. *Environmental Toxicology and Chemistry, 27*(8), 1735–1743.

Jover, R., Ponsoda, X., Castell, J. V., & Gómez-Lechón, M. J. (1994). Acute cytotoxicity of ten chemicals in human and rat cultured hepatocytes and in cell lines: Correlation between in vitro data and human lethal concentrations. *Toxicology in Vitro*, *8*(1), 47–54.

Kasprzak, K. S., Sunderman, F. W., & Salnikow, K. (2003). Nickel carcinogenesis. *Mutation research/fundamental and molecular mechanisms of mutagenesis, 533*(1), 67–97.

Kim, K.-H., Kabir, E., & Kabir, S. (2015). A review on the human health impact of airborne particulate matter. *Environment International, 74,* 136–143.

Lee, M. (2014). An analysis on the concentration characteristics of PM2.5 in Seoul, Korea from 2005 to 2012. *Asia-Pacific Journal of Atmospheric Sciences*, *50*(1), 585–594.

Lee, J.-C., Son, Y.-O., Pratheeshkumar, P., & Shi, X. (2012). Oxidative stress and metal carcinogenesis. *Free Radical Biology and Medicine, 53*(4), 742–757.

Lippmann, M., Ito, K., Hwang, J.-S., Maciejczyk, P., & Chen, L.-C. (2006). Cardiovascular effects of nickel in ambient air. *Environmental Health Perspectives, 114*(11), 1662.

Lu, S. C. (2009). Regulation of glutathione synthesis. *Molecular Aspects of Medicine, 30*(1), 42–59.

Ma, J. C., & Dougherty, D. A. (1997). The cation–π interaction. *Chemical Reviews, 97*(5), 1303–1324.

Mackay, D., Shiu, W.-Y., Ma, K.-C., & Lee, S. C. (2006). *Handbook of physical–chemical properties and environmental fate for organic chemicals*. Boca Raton: CRC Press.

Mahadevi, A. S., & Sastry, G. N. (2012). Cation–π interaction: Its role and relevance in chemistry, biology, and material science. *Chemical Reviews, 113*(3), 2100–2138.

Moorthy, B., Chu, C., & Carlin, D. J. (2015). Polycyclic aromatic hydrocarbons: From metabolism to lung cancer. *Toxicological Sciences, 145*(1), 5–15.

Muthusamy, S., Peng, C., & Ng, J. C. (2016a). The binary, ternary and quaternary mixture toxicity of benzo[a]pyrene, arsenic, cadmium and lead in HepG2 cells. *Toxicology Research, 5*(2), 703–713.

Muthusamy, S., Peng, C., & Ng, J. C. (2016b). Effects of binary mixtures of benzo[a]pyrene, arsenic, cadmium, and lead on oxidative stress and toxicity in HepG2 cells. *Chemosphere, 165,* 41–51.

Ng, K. M., Ma, N. L., & Tsang, C. W. (1998). Cation–aromatic π interaction in the gas phase: An experimental study on relative silver (I) ion affinities of polyaromatic hydrocarbons. *Rapid Communications in Mass Spectrometry, 12*(22), 1679–1684.

Perina, F. C., de Souza Abessa, D. M., Pinho, G. L. L., & Fillmann, G. (2011). Comparative toxicity of antifouling-compounds on the development of sea urchin. *Ecotoxicology*, *20*(8), 1870–1880.

Pope, C. A., III. (2000). What do epidemiologic findings tell us about health effects of environmental aerosols? *Journal of Aerosol Medicine, 13*(4), 335–354.

Pulido, M. D., & Parrish, A. R. (2003). Metal-induced apoptosis: Mechanisms. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 533*(1), 227–241.

Qu, X., Wang, X., & Zhu, D. (2007). The partitioning of PAHs to egg phospholipids facilitated by copper and proton binding via cation–π interactions. *Environmental Science and Technology, 41*(24), 8321–8327.

Ravindra, K., Mittal, A. K., & Van Grieken, R. (2001). Health risk assessment of urban suspended particulate matter with special reference to polycyclic aromatic hydrocarbons: A review. *Reviews on Environmental Health, 16*(3), 169–189.

Steenhof, M., Gosens, I., Strak, M., Godri, K. J., Hoek, G., Cassee, F. R., et al. (2011). In vitro toxicity of particulate matter (PM) collected at different sites in the Netherlands is associated with PM composition, size fraction and oxidative potential—The RAPTES project. *Particle and Fibre Toxicology, 8*(1), 26.

Stogiannidis, E., & Laane, R. (2015). Source characterization of polycyclic aromatic hydrocarbons by using their molecular indices: An overview of possibilities. In D. M. Whitacre (Ed.), *Reviews of environmental contamination and toxicology* (pp. 49–133). Berlin: Springer.

Tao, Y., Li, W., Xue, B., Zhong, J., Yao, S., & Wu, Q. (2013). Different effects of copper(II), cadmium(II) and phosphate on the sorption of phenanthrene on the biomass of cyanobacteria. *Journal of Hazardous Materials, 261,* 21–28.

Tao, Y., Xue, B., Yang, Z., Yao, S., & Li, S. (2015). Effects of metals on the uptake of polycyclic aromatic hydrocarbons by the cyanobacterium *Microcystis aeruginosa*. *Chemosphere, 119,* 719–726.

Valavanidis, A., Fiotakis, K., & Vlachogianni, T. (2008). Airborne particulate matter and human health: Toxicological assessment and importance of size and composition of particles for oxidative damage and carcinogenic mechanisms. *Journal of Environmental Science and Health, Part C, 26*(4), 339–362.

Van Leeuwen, H. (2000). Speciation dynamics and bioavailability of metals. *Journal of Radioanalytical and Nuclear Chemistry, 246*(3), 487–492.

Vijay, D., & Sastry, G. N. (2008). Exploring the size dependence of cyclic and acyclic π-systems on cation–π binding. *Physical Chemistry Chemical Physics, 10*(4), 582–590.

Voutsa, D., & Samara, C. (2002). Labile and bioaccessible fractions of heavy metals in the airborne particulate matter from urban and industrial areas. *Atmospheric Environment*, *36*(22), 3583–3590.

Wang, P., Luo, L., Ke, L., Luan, T., & Tam, N. F. Y. (2013). Combined toxicity of polycyclic aromatic hydrocarbons and heavy metals to biochemical and antioxidant responses of free and immobilized *Selenastrum capricornutum*. *Environmental Toxicology and Chemistry, 32*(3), 673–683.

WHO & UNAIDS. (2006). *Air quality guidelines: Global update 2005*. Geneva: World Health Organization.

Xiao, L., Qu, X., & Zhu, D. (2007). Biosorption of nonpolar hydrophobic organic compounds to *Escherichia coli* facilitated by metal and proton surface binding. *Environmental Science and Technology, 41*(8), 2750–2755.

Yang, L., Liu, G., Lin, Z., Wang, Y., He, H., Liu, T., et al. (2016). Pro-inflammatory response and oxidative stress induced by specific components in ambient particulate matter in human bronchial epithelial cells. *Environmental Toxicology, 31*(8), 923–936.

Zhang, Z., Chau, P. Y., Lai, H., & Wong, C. (2009). A review of effects of particulate matter-associated nickel and vanadium species on cardiovascular and respiratory systems. *International Journal of Environmental Health Research, 19*(3), 175–185.