Comparisons of IL-8, ROS and p53 responses in human lung epithelial cells exposed to two extracts of PM2.5 collected from an e-waste recycling area, China

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Abstract
To identify the different effects of organic-soluble and water-soluble pollutants adsorbed on PM2.5 (PM: particulate matter) released from e-waste (electrical/electronic waste) on inflammatory response, oxidative stress and DNA damage, interleukin-8 (IL-8), reactive oxygen species (ROS) and p53 protein levels were determined and compared in human lung epithelial A549 cells exposed to extracts of PM2.5 collected from two sampling sites in an e-waste recycling area in China. It is found that both extracts induced increases of IL-8 release, ROS production and p53 protein expression. The differences between the organic-soluble and water-soluble extracts were determined as of significance for ROS production (p < 0.05) and p53 protein expression (p < 0.01). The ROS production and p53 protein expression induced by the organic-soluble extracts were found to be greater than those induced by the water-soluble extracts, for both sampling sites. The results indicated that PM2.5 collected from the e-waste recycling areas could lead to inflammatory response, oxidative stress and DNA damage, and the organic-soluble extracts had higher potential to induce such adverse effects on human health.

Keywords: IL-8, ROS, p53, PM2.5, A549 cells, e-waste

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1. Introduction

Electrical/electronic waste (e-waste) is obsolete or end-of-life electrical/electronic goods, such as electromotors, transformers, computers, printers, copying machines, television sets, and mobile phones. Every year, 20–50 million tons of e-waste are generated worldwide, and a large part of this is exported to China for recycling (UNEP 2005).

Many pollutants have been released from e-waste into the environment as a consequence of the crude process of recycling of e-waste (Deng et al 2006, Fu et al 2008, Schmidt 2002, Wen et al 2008, Wong et al 2007) and they can accumulate...
in the human body through various pathways. Inhalation of contaminated air is believed to be one of the most important pathways. Many pollutants such as PCDD/Fs, PCBs, PBDEs, PAHs, cadmium, chromium, lead, and arsenic have been identified in atmospheric particles in and around the e-waste dismantling areas of China (Chen et al. 2009, Deng et al. 2006, Wen et al. 2008).

Epidemiological studies have revealed the association between exposure to ambient air PM, especially PM2.5, with increased mortality and morbidity for some diseases, such as respiratory and cardiovascular diseases (Brunekreef and Holgate 2002). The underlying mechanisms of action by which air pollution PM2.5 induces adverse health effects are of immense scientific interest. A widely accepted hypothesis is that the PM toxicity depends on their composition. Already, it is well known that air pollution PM is a complex mixture of chemical elements including various organic and inorganic components that can in fact interact and adsorb on condensation nuclei (Dagher et al. 2006). Many of the published studies on PM toxicity have been conducted using complex mixtures, while some have focused on one kind of chemical component such as the organic compounds. Both organic and inorganic pollutants have been found in atmospheric particles collected from the e-waste recycling areas in China. However, to our knowledge, few studies have compared the adverse effects of the two kinds of chemical components coated on PM2.5 in the e-waste recycling areas.

In the present study, PM2.5 was collected from Taizhou in Zhejiang province, which is one of major e-waste dismantling areas in China. In the area, e-waste has been ‘recycled’ (so-called) for decades. More than two million tons of e-waste have been dismantled in the area every year to recycle metals (Gu et al. 2010). It was estimated that more than 60 thousand people were involved in e-waste dismantling in Taizhou. The techniques used in the recycling of the e-waste are often primitive without the appropriate facilities to safeguard environmental and human health (Chen et al. 2009). These activities not only produce amounts of particulate matter, but also release various pollutants that can be adsorbed on the surface of PM.

In Taizhou, some pollutants have been detected in ambient air PM, especially in PM2.5. The average total concentration of 38 PCB congeners was determined as 1227 pg m$^{-3}$ in PM2.5 collected from Taizhou in winter (Han et al. 2010). PAHs and phthalates were detected at 248.5 and 326.6 ng m$^{-3}$, respectively, in PM2.5 in the same area in winter (Gu et al. 2010). Meanwhile, levels as high as 335 pg m$^{-3}$ for PBDEs in PM2.5 have also been reported in the same area in winter (Han et al. 2009).

It has been reported that exposure to PM2.5 induces oxidative damage and inflammatory responses in human lung epithelial cells (Dagher et al. 2005, Garcon et al. 2006). Oxidative stress and inflammatory responses have both been proven to damage DNA (Ryter et al. 2007) and lead to the activation of transcription factors such as p53 (Lu et al. 2006, Martindale and Holbrook 2002). In this study, levels of reactive oxygen species (ROS), interleukin-8 (IL-8), and p53 protein in human lung epithelial A549 cells were determined, to compare the different responses for the organic-soluble and water-soluble extracts of PM2.5 collected from the e-waste recycling area as regards oxidative damage, inflammatory responses and p53 expression.

2. Materials and methods

2.1. Sample collection and extraction

In order to regulate the e-waste dismantling activities and control the pollutant emissions, an e-waste dismantling industrial park was set up near the small town of Taizhou by the local government in 2004. Two sampling sites, FJ and JK, were selected near the dismantling industrial park, located on the rooftops of a four-story building and a six-story building, respectively. Both sampling sites were located downwind of the dismantling industrial park and the sampling site JK was closer to the park. The two sampling sites were about 2 km apart.

PM2.5 samples were collected simultaneously using two high-volume samplers (Graseby, GMWT 2200) at the two sampling sites over the period 11–25 October 2007. During this period, the diurnal air samples were collected from about 9:00 and the sampling time was nominally 24 h. The flow rate was set at 1.1 m$^3$ min$^{-1}$ and the air volume collected was calculated. Calibrations were made before and after each sampling, and the average value was used for the calculation of the air volume collected. The PM2.5 samples were collected on quartz fibers (Whatman, QM-A 20). Prior to use, the fibers were baked at 550 ℃ for 4 h. Samples were stored in 250 ml glass bottles in a refrigerator with 3–5 ml methylene chloride added to prevent microbial growth. Meteorological data and sample collection data are shown in supplementary data table S1 (available at stacks.iop.org/ERL/6/024013/mmedia).

Organic-soluble and water-soluble pollutants were separately extracted from the fibers. For the organic-soluble pollutants, half of each fiber was Soxhlet-extracted with an acetone-hexane mixture (1:1) for 24 h. After extraction, the extract was cleaned on an anhydrous Na$_2$SO$_4$ column with hexane and then concentrated by rotary evaporation. The extract was then evaporated to near dryness under a gentle N$_2$ gas flow. The fraction of organic-soluble pollutants was adjusted to 50 µl using dimethyl sulfoxide for the following **in vitro** exposure. For the preparation of water-soluble pollutants, a quarter of each fiber was ultrasonically extracted three times with a total volume of 16 ml water. The extracts from the three extractions were pooled and sterilized through a filter unit (MillexGP, Millipore). A blank fiber was also extracted following the above procedures as a control.

2.2. Exposure tests

Human lung epithelial cells (A549) were grown in DMEM medium containing 5% fetal calf serum and incubated at 37 ℃ in a humidified 5% CO$_2$ incubator. In the following exposure tests, the concentration of each extract of organic-soluble or water-soluble pollutants was 1 µg m$^{-3}$ in the medium without fetal calf serum (1 ml of medium contains organic-soluble or
Figure 1. IL-8 release, ROS production and p53 protein expression in A549 cells exposed to the organic-soluble and water-soluble extracts from both sampling sites, FJ and JK. ■: the organic-soluble extracts; ▲: the water-soluble extracts. Nonparametric tests of two independent samples indicate significant differences in ROS production ($p < 0.05$) and p53 protein expression ($p < 0.01$) between the organic-soluble and water-soluble extracts from the two sampling sites.

2.3. IL-8 and p53 expression

A549 cells were seeded in 12-well plates at a density of $1 \times 10^5$ cells/well. Following cell attachment, the medium was removed and the cells were exposed to 1 ml of medium containing the extract of organic-soluble or water-soluble pollutants for 24 h. After exposure, the medium was collected for IL-8 analysis. The cell monolayer was washed twice with PBS and then lysed with RIPA lysis and extraction buffer (Biosciences) at 4°C for 15 min. The concentration of p53 protein was determined in the lysis solution. IL-8 and p53 protein were quantitatively analyzed by using IL-8 ELISA (BioLegend) and p53 ELISA kits (R&D Systems), respectively, following the manufacturer’s instructions. The total protein concentrations were also determined in the medium and lysis solutions.

2.4. The ROS assay

Cells were seeded in 96-well plates at a density of $2 \times 10^4$ cells/well. After the cells attached, the medium was removed and the cells were exposed to 100 μl of medium containing the extract of organic-soluble or water-soluble pollutants for 24 h. After exposure, the medium was removed and the cells were washed twice with PBS. ROS within the cells were detected using a ROS assay kit (Beyotime Institute of Biotechnology), following the manufacturer’s instructions.

2.5. Statistical analysis

The experimental data were expressed as the mean ± SD. Differences between the control and the samples were assessed with independent sample T-tests. Nonparametric tests of two independent samples were employed to analyze the differences of various response endpoints to the two extracts, using the Mann–Whitney U test. Linear regression analysis was used to evaluate the relationships among the different parameters. Statistical analysis was performed using SPSS 16.0. Differences were considered significant when $p < 0.05$.

3. Results

The responses of IL-8, ROS and p53 protein are shown in figure 1 and supplementary data table S2 (available at stacks.iop.org/ERL/6/024013/mmedia). At both sampling sites, significant increases of IL-8 were observed in A549 cells exposed to most of the organic-soluble and water-soluble extracts of PM2.5. The concentrations of IL-8 in the cells exposed to the organic-soluble and water-soluble extracts of PM2.5 from sampling site FJ were determined as ranging from 42.22 to 136.14 and 0.24 to 58.8 ng mg$^{-1}$ of protein, respectively. In
Figure 2. Comparison of average responses of IL-8, ROS and p53 protein in A549 cells exposed to both the organic-soluble and water-soluble extracts of PM2.5 from sampling sites FJ and JK.

contrast with the results for sampling site FJ, the average rate of increase of IL-8 induced by the organic-soluble extracts of PM2.5 collected from sampling site JK was not significantly higher than that induced by the water-soluble extracts ($p > 0.05$).

The ROS production induced by the organic-soluble and water-soluble extracts of PM2.5 collected from sampling site FJ ranged from 53.40 to 101.60 A (absorbance units) and from 75.14 to 126.32 A, respectively. For sampling site JK, the ROS production ranged from 65.22 to 129.99 A for the organic-soluble extracts and from 102.33 to 174.70 A for the water-soluble extracts. Unlike the case for the controls, the rates of increase of ROS production induced by the organic-soluble extracts were higher than those induced by the water-soluble extracts on most of the sampling dates for both sampling sites. The average rates of increase of ROS production as compared against those for the controls were calculated as 145.43 and 119.37% for the organic-soluble and water-soluble extracts of PM2.5 collected from sampling site FJ, and 198.16 and 157.61% for the organic-soluble and water-soluble extracts of PM2.5 collected from sampling site JK. Statistical analysis revealed that the differences in ROS production between the organic-soluble and water-soluble extracts were significant for both sampling sites ($p < 0.05$).

The concentrations of p53 protein were detected as ranging from 19.50 to 162.00 pg mg$^{-1}$ of protein for the organic-soluble extracts and from 62.09 to 195.85 pg mg$^{-1}$ of protein for the water-soluble extracts at sampling site FJ. The average rates of increase of p53 protein were 579.68 and 123.55% compared to those of the controls for the organic-soluble and water-soluble extracts at the sampling site, respectively. At sampling site JK, the concentrations of p53 protein and their average rate compared to those for the controls were determined as ranging from 61.14 to 163.14 pg mg$^{-1}$ of protein and as 669.20% for the organic-soluble extracts, and as ranging from 91.27 to 193.79 pg mg$^{-1}$ of protein and as 156.43% for the water-soluble extracts. Moreover, the expression of p53 protein induced by the water-soluble extracts was much less than those induced by the organic-soluble extracts for both sampling sites ($p < 0.01$).

The average responses of IL-8, ROS and p53 protein in A549 cells exposed to PM2.5 from sampling site JK were all found to be higher than those for the cells exposed to PM2.5 from sampling site FJ for both the organic-soluble and water-soluble extracts with a range of 15–70% (figure 2). Furthermore, the increases of IL-8 and ROS were similar for the two sampling sites. However, the responses of p53 protein in the cells exposed to the organic-soluble extracts were much higher than those for the cells exposed to the water-soluble extracts at the two sampling sites.

4. Discussion

Many studies have reported the induction of IL-8 and ROS in human respiratory epithelial cells exposed to particulate matter (Becker et al 2005, Fujii et al 2001, Garcon et al 2006, Jiménez et al 2002, Ryter et al 2007). The increase of IL-8 release and ROS production found in the present study indicated an inflammatory response and oxidative stress in A549 cells induced by PM2.5. Meanwhile, the induction of p53 protein detected also showed the potentials for DNA damage. The results revealed a possible health risk to local residents through inhalation of PM2.5.

It is well known that inflammatory responses and oxidative stress can lead to DNA damage and consequently activate p53 protein expression to facilitate DNA repair. Therefore, there may be a correlation between IL-8 release or ROS production and p53 protein expression in A549 cells exposed to the extracts of PM2.5 collected from the e-waste recycling area. However, statistical analysis did not find such correlations. The results revealed that IL-8 release and ROS production were not the only causes of p53 protein expression. Inflammatory responses can in fact generate numerous growth factors, cytokines, and reactive species that may cause DNA damage, besides IL-8 (Coussens and Werb 2002, Quay et al 1998).
Oxidative stress can arise through both reactive oxygen and nitrogen species (Coussens and Werb 2002). Therefore, p53 protein expression in the cells could also originate from other factors undetected in the present study.

In the present study, the components adsorbed on PM2.5 were divided into two fractions, namely organic-soluble and water-soluble extracts, to compare their different effects on IL-8 release, ROS production and p53 protein expression in A549 cells. Both extracts were found to induce IL-8 release, ROS production and p53 protein expression in the cells. Differences in response of the three biomarkers were found between the two extracts. No significant variation of IL-8 release was observed between the organic-soluble and water-soluble extracts for both sampling sites. However, the difference was determined as significant between the two extracts for ROS production ($p < 0.05$) and p53 protein expression ($p < 0.01$) for both sampling sites. The results indicated that the organic-soluble and water-soluble extracts can lead to different responses of ROS production and p53 protein expression rather than IL-8 release, and the constituents of the organic-soluble extracts were more likely to induce oxidative stress and DNA damage than those of the water-soluble extracts.

The particle toxicity is believed to be attributable to their composition. The constituents including organic-soluble and water-soluble pollutants have been detected in PM in the e-waste recycling area in previous studies (Chen et al. 2009, Deng et al. 2006, Wen et al. 2008). It is reported that, among the biologically active components adsorbed on PM, organic compounds such as PAHs seem to play an especially important role in the inflammatory response and oxidative stress (Baulig et al. 2004, Bonvallot et al. 2001). Meanwhile, many studies have demonstrated that soluble PM components, especially metals, also contribute to inflammatory responses and oxidative stress induced by particulate matter including copper and iron (Kennedy et al. 1998, Zhang et al. 2008).

In addition, for both the organic-soluble and the water-soluble extracts, the responses of IL-8 release and ROS production were found to be higher in the cells exposed to PM2.5 from sampling site JK than in those cells exposed to PM2.5 from sampling site FJ. These results were consistent with the heavier air pollution observed at sampling site JK, resulting from the site being located downwind of and closer to the dismantling industrial park.

Although the risk of inhalation of polluted air on human health has not yet been evaluated in the e-waste dismantling area, the results obtained in the present study have revealed the potential for an impact on human health arising from inhalation of such polluted air in the area. Most of the dismantling workers and local residents lived in or around the dismantling industrial park. They were incessantly exposed to polluted air, without any protection. An unpublished survey has revealed that diseases such as cardiovascular diseases and various cancers have become more common during the past few decades in the area. Therefore, further studies are urgently required to identify the association between airborne pollutants and the impacts on human health in these e-waste dismantling areas.

5. Conclusions

IL-8 release and ROS production detected in A549 cells indicated inflammatory responses and oxidative stress induced by PM2.5 collected from the e-waste recycling area. p53 protein expression suggested potential DNA damage induced by the PM2.5 samples within the cells. Comparing the different responses of IL-8 release, ROS production and p53 protein expression for the organic-soluble and water-soluble extracts, the organic-soluble extracts of PM2.5 showed higher potential to induce adverse effects than the water-soluble extracts. The species and concentrations of pollutants were not chemically determined in either of the extractions of the PM2.5 in the present study. Therefore, the components of both extracts and the main contributors to such adverse effects should be determined for PM2.5 in the area in further study.

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