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Decreased vaccine antibody titers following exposure to multiple metals and metalloids in e-waste-exposed preschool children☆

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ABSTRACT

We explored acquired immunity resulting from vaccination in 3 to 7-year-old children, chronically exposed to multiple heavy metals and metalloids, in an e-waste recycling area (Guiyu, China). Child blood levels of ten heavy metals and metalloids, including lead (Pb), arsenic (As), mercury (Hg), chromium (Cr), cadmium (Cd), manganese (Mn), nickel (Ni), copper (Cu), zinc (Zn) and selenium (Se), and seven vaccine antibodies (diphtheria, pertussis, tetanus, hepatitis B, Japanese encephalitis, polio, measles) were measured. The exposed group had higher levels of blood Pb, Mn, Cu, Zn and Cr compared to the reference group ($P < 0.05$). Levels of all vaccine antibodies in the exposed group were significantly lower than in the reference group ($P < 0.01$). All vaccine antibodies negatively correlated with blood concentrations of Cu, Zn and Pb, based on spearman rank correlation analysis. Multiple logistic regression and univariate analyses identified the location of residence (Guiyu), high blood Pb (>10 μg/dL) and high blood Cu and Zn (upper median value of each group) to be inversely associated with seven antibody titers. Antibody titers increased with age, BMI, high blood Mn (>15 μg/L), and high blood Cd and Ni (upper median value of each group). Results suggest multiple heavy metal and metalloid exposure, especially to Pb, Zn and Cu, may be a risk factor inhibiting the development of child immunity, resulting in decreased child antibody levels against vaccines.

1. Introduction

Due to the increasing global demand for electrical and electronic products, and the short lifespan of these products, e-waste continues to be a rapidly growing worldwide environmental problem (Heacock et al., 2016), with a large amount of e-waste from developed countries being shipped for disposal and recycling to developing countries, such as China, India and Nigeria (Awasthi et al., 2016; Lundgren, 2012). For example, Guiyu township, located in the Guangdong province of southern China, has been involved in e-waste recycling for more than 30 years, and is one of the largest e-waste processing centers in China (Brigden et al., 2005). Heavy metals and metalloids can be found in e-waste, including Pb, Cu, Cr, Mn, Ni, Hg, Cu, As, and Zn, many of which are potentially, or known to be, hazardous (Heacock et al., 2016; Song and Li, 2015). In the process of e-waste dismantling and recycling, involving activities such as sorting, open burning, and acid leaching, fly ash particulates containing heavy metals, such as Pb, Cu, Zn, Hg, Cd and heavy metalloids (As, Sb), are emitted into the environment, and epidemiological studies indicate higher levels of heavy metals in the air (Zeng et al., 2016b), soil, road dust and water surrounding e-waste dismantling locations (Alabi et al., 2012; Huo et al., 2007; Leung et al., 2008; Yekeen et al., 2016; Zhang et al., 2014).

Heavy metals and metalloids enter the human body through oral intake, inhalation, dermal contact, and hair (Eastman et al., 2013; Paolo et al., 2011; Wang et al., 2009), to cause acute and chronic toxicity, such as damage to blood components, lungs,

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kidney, liver, and the peripheral nervous, central nervous and immune systems, occasionally resulting in lethality (Vallverdu-Coll et al., 2015b; Zeng et al., 2016a). Our previous studies show that children in Guiyu have a significantly higher heavy metal content (Pb, Cd, Cr) than reference groups (Xu et al., 2013; Yang et al., 2013; Zeng et al., 2016b; Zheng et al., 2013), which is of particular concern, compared with adults, since children are more susceptible to heavy metals and metalloids (Guney and Zagury, 2014). Studies have shown that heavy metals and metalloids affect the human immune system. Pb increases the secretion of pro-inflammatory cytokines, such as IL-6 and TNF (Gillis et al., 2012), and prolonged exposure to lead nitrate causes significant decreases in lymphocyte and monocyte levels (Sharma et al., 2010). Our previous cross-sectional results show elevated Pb levels associate with the lower percentages of NK cells and altered platelets, IL-1β and IL-27 in preschool children (Zhang et al., 2016). Li et al. found a potential link between exposure to pollutants from e-waste recycling and both elevated oxidative stress and altered generation of reactive oxygen species (ROS) levels by white blood cells, suggesting potential ROS-related health effects for residents at e-waste sites (Li et al., 2013). Pb can also affect both cell-mediated and antibody-mediated immunity, and decrease total serum antibody levels in rats (Heo et al., 1997; Mishra et al., 2006). Low concentrations of mercuric chloride will affect immune cells and increase susceptibility to infectious diseases (Gardner et al., 2010) and inorganic and organic Hg can increase the release of inflammatory factors (Gardner et al., 2009). Ni is a ubiquitous metal that is responsible for allergic skin reactions in some cases, and chronic arsenic exposure can influence the immune response by inducing alterations in lung gene and protein expression (Kozul et al., 2009). Our recent studies demonstrate a negative association between blood lead and antibody levels, against hepatitis B surface antigen, lower antibody titers against measles, mumps, and rubella (MMR) vaccination with higher Blood Pb levels in children from Guiyu (Lin et al., 2016; Xu et al., 2015). The ability of heavy metals and metalloids to inhibit immune responses suggests that heavy metal and metalloid exposure could exert profound effects on vaccine antibody levels, and thus result in broad susceptibility to a variety of infections.

In 2005, a central government regulation on vaccine distribution and vaccination divided the country's approved vaccines into two categories. The first category requires residents to be vaccinated, but at no cost to the residents. They can help improve oversight of these vaccines to better protect the health of children in China. The vaccines we study belong to the first category. According to the national childhood immunization schedule of the People's Republic of China, children are required to be vaccinated against diphtheria, pertussis and tetanus (all administered at three-, four-, five-and 18-months of age), hepatitis B (administered at birth, one- and six-months of age), Japanese encephalitis (inoculated at eight-and 24-months of age), polio (inoculated at two-, three and four-months of age), and measles (inoculated at eight-and 18-months of age). Understanding the susceptibility of children living in e-waste areas, following vaccination, is necessary to prevent and control infectious disease. However, the associations between multiple heavy metals (except Pb) and metalloids and the levels of antibody titers, following vaccination, have yet to be reported in children from e-waste areas. In this study, we measured the concentrations of multiple heavy metals and metalloids, and levels of antibodies against vaccinated agents, including diphtheria, pertussis, tetanus, hepatitis B, Japanese encephalitis, polio, and measles, in 3- to 7-year-old preschool children. The aim of this study is to determine the association between heavy metal and metalloid concentrations and vaccine antibody levels in order to identify whether exposure to multiple heavy metals and metalloids affects child immunity.

2. Materials and methods

2.1. Study population and sample collection

A total of 284 children, 3- to 7-years of age, residing in Guiyu (n = 157) and Haojiang (n = 127), were recruited in 2012. The sampling location Guiyu was previously described (23°327′N, 116°342′E) (Wu et al., 2010). Haojiang is located about 50 km east of Guiyu, and was used as a reference area (23°17′N, 116°43′E). The main industry in Haojiang is fishing, with no e-waste recycling work being performed there (Fig. 1). The populations in these two areas have similarities in cultural background, lifestyle and socio-economic status. Detailed explanations of the study and questionnaires were administered to the children's parents or guardians. The questionnaire addressed factors that might influence child heavy metal and metalloid levels in the blood, such as the residence, physical status, dietary habits, nutrition and work environment of the parents. A medical and health history, including vaccination history, respiratory symptoms (wheeze, cough, dyspnea, phlegm) and allergic disease (such as asthma), was also taken into consideration.

After informed consent was obtained from the parents or guardians, a total of 2 ml venous blood was collected by trained nurses, placed on ice and stored at −20 °C until analysis. Physical indices (height and weight, and head and chest circumference) were also measured. To avoid heavy metal and metalloid contamination, all plastic tubes and containers used for collection of blood samples were pre-washed, soaked in dilute nitric acid (HNO_3) and rinsed with deionized water. This study protocol was approved by the Human Ethics Committee of Shantou University Medical College.

2.2. Levels of heavy metals and metalloids in blood samples analysis by inductively coupled plasma mass spectrometry (ICP-MS)

One mL of blood was digested with five mL of 68% HNO_3 in a microwave oven (XT-III, Shanghai Xintuo, China) for 2.5 min. After digestion, ultrapure water was added to a final volume of ten mL. The concentrations of Zn, Cu, Se, Mn, Ni, Pb, As, Hg, Cr and Cd were determined by ICP-MS (Agilent 7700× ICP-MS, USA) (Moeller et al., 2007). An internal standard comprised of a mixture of Bi (Bismuth), In (Indium), Re (Rhenium) and Sc (scandium), all at a final concentration of 10 mg/L, was added on-line to all samples and standard solutions.

2.3. Detection of the levels of seven vaccine antibodies by ELISA

Antibody levels in serum were measured by the relevant enzyme-linked immunosorbent assay (ELISA) kit (R&D systems, USA), using an enzyme microplate reader (Labsystems Multiskan MS 352, Finland) and microplate washer (Thermo Labsystems AC8, Finland). Briefly, solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle is performed. The wells are coated with antigen, and specific antibodies of samples binding to the antigen coated wells are detected by a secondary enzyme conjugated antibody (E-Ab) specific for human IgG. After the substrate reaction, the intensity of the developed color is proportional to the amount of IgG-specific antibodies to be detected. Results of samples can be quantified directly according the standard curve, for instance, the Tetanus IgG (IU/mL) (Supplemental Fig. 1).