Ecotoxicology and Environmental Safety 163 (2018) 674–684

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Lead, cadmium, arsenic, and mercury combined exposure disrupted synaptic homeostasis through activating the Snk-SPAR pathway

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ARTICLE INFO

Keywords:
Metal mixture
Synergistic neurotoxicity
Synaptic homeostasis
Snk-SPAR pathway
Human environmental exposure levels

ABSTRACT

Lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg) are among the leading toxic agents detected in the environment, and they have also been detected simultaneously in blood, serum, and urine samples of the general population. Meanwhile early neurologic effects and multiple interactions of Pb, Cd, As, and Hg had been found in children from environmentally polluted area. However, the current studies of these four metals were mostly limited to the interactions between any two metals, whereas the interaction characteristics between any three and four metals were rarely studied. In our study, we firstly explored the characteristics of the neurotoxic interactions among these four elements in nerve cells with factorial designs. The results showed that Pb + Cd + As + Hg co-exposure had a synergistic neurotoxic effect that was more severe than that induced by any two or three metals, when their individual metals were at human environmental exposure (in the blood of U.S. population) relevant levels and below no observed adverse effect levels (NOAELs). Therefore, Pb + Cd + As + Hg co-exposure at human environmental exposure relevant levels were further selected to examine synaptic homeostasis as the cellular and molecular foundation of learning and memory. We reported for the first time that Pb + Cd + As + Hg co-exposure induced dose-dependent decreases of the dendritic lengths and branching, as well as spine density and mature phenotype in primary hippocampal neurons, and the stimulated neurite outgrowths in NGF-differentiated PC12 cells. And the above synaptic homeostasis disruption was associated with serum induced kinase (Snk)-spine associated Rap GTPase activating protein (SPAR) pathway. Our study suggests that human environmental Pb, Cd, As, and Hg co-exposure has the potential to evoke synergistic neurotoxicity even if their individual metals are below NOAELs, which reinforces the need to control and regulate potential sources of metal contamination.

1. Introduction

Lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg) were the only four heavy metals among the ten chemicals of major public health concern released by the World Health Organization (WHO) (WHO, 2010), and they have been detected simultaneously in blood, serum, and urine samples of the U.S. population from the National Health and Nutrition Examination Survey (NHANES) as well as Chinese population reported by China's Centres for Disease Control (CDC) (CDC (Centers for Disease Control and Prevention), 2015; Ding et al., 2014a, 2014b; Wu et al., 2013). These four metals have been widely utilized in industry and daily life. The accumulation of these environmental pollutants in ecosystems is a major source for human exposure and hence poses a threat to human health (Olmedo et al., 2013; Golding et al., 2013; Zhao et al., 2014). Governments all over the world have taken measures to reduce metal pollution, with several notable achievements (Selin, 2018; Hu et al., 2014b; CDC, 2015). However, further biodegradation of these four metals is extremely difficult, resulting in long lives after their release into the environment. Therefore, the long-term and low-level exposure of these four metals has gained attention.

A cohort study concerning the influence of low-level lead exposure on school performance among children in Chicago Public Schools

Abbreviations: As, arsenic; BIC, bicuculline; Cd, cadmium; Hg, mercury; MM, metal mixture; Mut, mutation type; NGF, nerve growth factor; NOAELs, no observed adverse effect levels; Pb, lead; PSD, postsynaptic density; ROS, reactive oxygen species; Snk, serum induced kinase; SPAR, spine associated Rap GTPase activating protein; Wt, wild type; $[Ca^{2+}]i$, intracellular free calcium ion concentration

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https://doi.org/10.1016/j.ecoenv.2018.07.116
Received 24 April 2018; Received in revised form 19 July 2018; Accepted 28 July 2018

indicated that blood lead concentrations below 10 μg/dL (even below 5 μg/dL) were inversely associated with reading and math scores in 3rd-grade children (Evens et al., 2015). The anthropometric effects of low-level prenatal mercury exposure in northern China suggest that prenatal Hg exposure could attenuate fetal and infant growth, with the geometric mean level of maternal blood Hg at 2.29 μg/L (Ou et al., 2015). A large survey conducted in five cadmium-polluted areas revealed that the overall benchmark dose lower confidence limits (BMDLs) of urinary Cd for urinary β2-microglobulin with an excess risk of 10% were 2.00 μg/g creatinine (μg/g cr) and 1.69 μg/g cr in males and females respectively, which were significantly lower than the WHO threshold level of 5 μg/g cr for Cd-related renal effects (Ke et al., 2015). The health impairments caused by long-term exposure to low levels of single metals deserve great concern. However, human exposure to environmental chemicals is most characterized as exposure to mixtures (CDC, 2004). Thus, studies on combined exposure are more important and more difficult than single-metal-exposure studies.

A cross-sectional European survey in 8.5–12.3 years of age children from environmentally polluted area showed the multiple interactions of Pb, Cd, As, and Hg at environmental exposure levels (de Burbure et al., 2006). The possible interactions between two metals were analyzed using two-way analysis of variance. Multiple interactions had been found such as, an interaction between blood Pb and urinary Hg increasing serum creatinine, and urinary Hg exacerbates the increase in the end products of dopamine metabolism urinary homovanillic acid linked to blood Cd. However, the current study of these four metals were mostly limited to the interactions between any two metals with relatively high exposure in environmentally polluted area, whereas the interaction characteristics between any three and four of these metals were rarely studied. Thus, there is an extremely necessary to elucidate the possible interactions among these four heavy metals at low levels in neurotoxicity.

Synaptic homeostasis has often been argued as the cellular and molecular foundation of learning and memory (Blackwell et al., 2018; Herzer et al., 2018; Howland and Wang, 2008). The stable synaptic connections can process, transmit and retain information in the neurons. However, the synapses are highly dynamic plasticity, which is due to the rearrangement of a specialized structure at many synapses in the dendritic spine (Meyer and Brose, 2003). Within the dendritic spines, there are numerous signaling molecules, cytoskeletal proteins, membrane receptors and scaffolding proteins located in the postsynaptic density (PSD). Spine-associated Rap GTPase-activating protein (SPAR) is a large multimodular scaffold protein in the PSD, which can combine with a complex of postsynaptic density 95 (PSD-95) and the N-methyl-D-aspartate (NMDA) receptors to regulate spine morphogenesis (Pak et al., 2001). Thus, it's an important candidate for remodeling of synapses (de Bartolomeis and Fiore, 2004). SPAR is confirmed to be phosphorylated by serum-induced kinase (Snk) and then be degraded by the ubiquitin proteasome system (UPS), which induce the depletion of SPAR from the spines. Then the rearrangement of spine structure causes the destabilization of synaptic connections (Fig. 1) (Pak and Sheng, 2003). Snk is up-regulated by neuronal activity and subsequently mediates the phosphorylation of SPAR, suggesting that Snk-SPAR signaling pathway represent a homeostatic regulatory process that disturbs synaptic connections in response to neuronal activity (Sui et al., 2017). Hence, we further checked whether Pb, Cd, As, and Hg combined exposure induce the rearranging of spine structure and destabilizing of synaptic connections, as well as the possible mechanism Snk-SPAR pathway involved.

To address some of this issue, we firstly explored the characteristics of the interactions among Pb, Cd, As, and Hg at low levels and selected the exposure doses and combinations of the metal mixture (MM) in factorial designs. Then investigated the synaptic structural alterations induced by Pb, Cd, As, and Hg combined exposure and assessed the contributions of Snk-SPAR pathway. Overall, the present study aims at exploring the synergistic neurotoxic effects of Pb, Cd, As, and Hg combined exposure at low doses, and identifying the mechanism involved in the disrupted synaptic homeostasis.

2. Materials and methods

2.1. Chemicals

Lead acetate (CAS number 6080-56-4) and cadmium chloride (CAS number 10108-64-2) were obtained from Sigma (St. Louis, MO, USA). Mercuric chloride (CAS number 7487-94-7) and sodium arsenite (CAS number 7784-46-5) came from Merck (Darmstadt, Germany). These heavy metals were dissolved in distilled water at a concentration of 10 mM as stock solutions respectively. In order to ensure the solubility and stability of heavy metal solutions, 0.1% acetic acid was added to help dissolve lead acetate during chemical solution preparation, and then the solution was protected from light to avoid the decomposable of mercuric chloride. Besides, the prepared chemical solutions were stored in teflon tube at 4 ℃ to keep off the adhesion of heavy metals.

2.2. Cell culture and NGF stimulation for PC12 cell

Primary hippocampal neurons were cultured following our previously described method (Fan et al., 2013). The human neuroblastoma (SH-SY5Y) cell line (cat. no. CRL-2266) and rat adrenergic neural tumor pheochromocytoma (PC12) cell line (cat. no. CRL-1721) were originally from the American Type Culture Collection (ATCC) and were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS, Gibco, USA) and 5% horse serum (HS) at 37 °C and 5% CO_2 with a humidified incubator (Heraeus, Germany).

For nerve growth factor (NGF) -stimulated differentiation studies (Greene and Tischler, 1976), PC12 cells were seeded on 12-well plates (3×10^4 cells) or cover slips coated with 0.1% poly-L-lysine in complete medium. The next day, the complete medium was replaced by differentiation medium comprising DMEM medium with 5% FBS supplemented with 50 ng/mL NGF (Gibco, Grand Island, NY, USA) (Kim et al., 2018; Kubota et al., 2017; Yu et al., 2016). The PC12 cells were stimulated to form a sympathetic neuron-like phenotype with neurite outgrowth for 3 days, and then treated with MM for another 3 days.

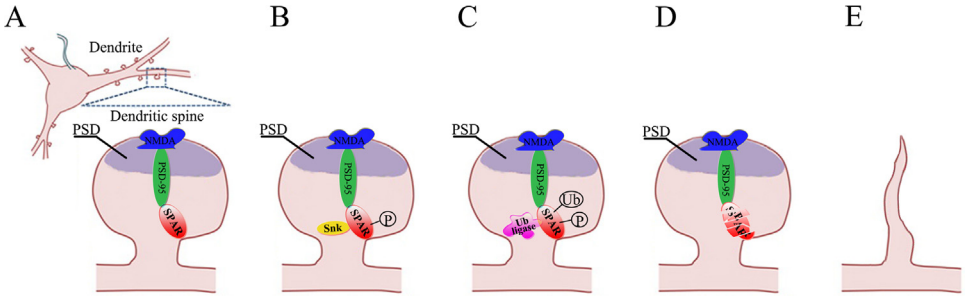

Fig. 1. Snk-SPAR pathway and spine destabilization. (A) In dendritic spine from neuronic dendrite, SPAR (red) forms a complex with PSD-95 (green) and NMDA receptor (blue), and associates with actin cytoskeleton (pink). (B) Snk (orange) is activated. Snk associates with and phosphorylates (P) SPAR. (C) An ubiquitin ligase (purple) recognizes phosphorylated SPAR and modifies it with ubiquitin (Ub). (D) SPAR is then degraded via the proteasome pathway. (E) The dendritic spine loses other protein components, and changes its shape to become a long, thin protrusion (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).