Chemosphere 132 (2015) 79–86

Contents lists available at ScienceDirect

Chemosphere

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

Low concentration toxic metal mixture interactions: Effects on essential and non-essential metals in brain, liver, and kidneys of mice on sub-chronic exposure

Samuel J. Cobbina [a], Yao Chen [a], Zhaoxiang Zhou [b], Xueshan Wu [b], Weiwei Feng [c], Wei Wang [c], Guanghua Mao [a], Hai Xu [a], Zhen Zhang [a], Xiangyang Wu [a,*], Liuqing Yang [b,*]

[a] School of the Environment, Jiangsu University, Xuefu Rd. 301, Zhenjiang 212013, Jiangsu, China
[b] School of Chemistry and Chemical Engineering, Jiangsu University, Xuefu Rd. 301, Zhenjiang 212013, China
[c] School of Food and Biological Engineering, Jiangsu University, Xuefu Rd. 301, Zhenjiang 212013, Jiangsu, China

HIGHLIGHTS

- Low dose metal mixtures interacted with toxic and essential metals.
- Interaction within metal mixtures were largely synergistic.
- Low dose exposures influenced homeostasis of toxic and essential metals.
- Essential metals in liver were influenced most by low dose metal mixtures.
- Elevation in toxic metals were linked to reductions in essential metals.

ARTICLE INFO

Article history:
Received 19 October 2014
Received in revised form 27 February 2015
Accepted 9 March 2015

Handling Editor: A. Gies

Keywords:
Interaction
Essential metals
Metal mixtures
Synergistic
Multivariate

ABSTRACT

The deleterious effects of long term exposure to individual toxic metals in low doses are well documented. There is however, a paucity of information on interaction of low dose toxic metal mixtures with toxic and essential metals. This study reports on interactions between low dose mixtures of lead (Pb), mercury (Hg), arsenic (As) and cadmium (Cd) and toxic and essential metals. For 120 d, six groups of forty mice each were exposed to metal mixtures, however, the control group was given distilled water. Exposure to Pb + Cd increased brain Pb by 479% in 30 d, whiles Pb + Hg + As + Cd reduced liver Hg by 46.5%, but increased kidney As by 130% in 30 d. Brain Cu, increased by 221% on Pb + Hg + As + Cd exposure, however, liver Ca reduced by 36.1% on Pb + Hg exposure in 60-d. Interactions within metal mixtures were largely synergistic. Principal component analysis (PCA) showed that low dose metal exposures influenced greatly levels of Hg (in brain and liver) and As (brain). The influence exerted on essential metals was highest in liver (PC1) followed by kidney (PC2) and brain (PC3). Exposure to low dose metal mixtures affected homeostasis of toxic and essential metals in tissues of mice.

1. Introduction

High loads of toxic elements are released into the environment each year from anthropogenic sources such as mining, smelting, and agriculture. Toxic metals such as lead (Pb), mercury (Hg), cadmium (Cd) and arsenic (As) beside existing individually, are also found as mixtures in various parts of the ecosystem (Liu et al., 2014). Of recent concern is their occurrence in low dose mixtures (Kortenkamp, 2014).

Exposure to toxic metals is deleterious to humans and occur through accumulation in the environment, with consumption of food and water as the main exposure pathways (Wahsha et al., 2014). Toxic metals like Pb, Hg, As and Cd are associated with disease conditions such as hypertension, neurological disorders, cognitive impairments, cerebral palsy, blindness, dysarthria, cancers, cirrhosis and hyperkeratosis (Boucher et al., 2014; He et al., 2014). Toxic trace metals do not have any specific role in an organism but are toxic, persistent and prefer to accumulate through consumption of food (García-Barrera et al., 2012). Essential metals

* Corresponding authors. Tel./fax: +86 511 88791200 (X. Wu). Tel./fax: +86 511 88791800 (L. Yang).
E-mail addresses: wuxy@ujs.edu.cn (X. Wu), yangliuqing@ujs.edu.cn (L. Yang).

http://dx.doi.org/10.1016/j.chemosphere.2015.03.013
0045-6535/

such as Cu, Mg, Ca, Zn and Fe are, however, needed for important metabolic functions in an organism (Provencher et al., 2014).

Studies show that exposure to individual toxic metals interact with toxic and essential metals in various tissues (Diacomanolis et al., 2014). However, there is a paucity of information, on the interaction of low dose toxic metal mixtures with toxic and essential metals. The few available studies were conducted using high dose mixtures (Mahaffey et al., 1981; Liu et al., 2000). Furthermore, available low dose studies, were conducted using the Lowest Observed Effect Levels (LOEL) of the individual metals. Employing the LOEL, Whittaker et al. recorded significant increases in kidney Cd and Pb of rats exposed to Pb + As (90-d) and Cd + As (180-d), respectively. In their study, exposure to Cd + As increased kidney Fe and Cu in 90-d (Whittaker et al., 2011).

Fortoul et al. assessed the concentration of Pb and Cd in the lungs of mice exposed to Pb + Cd (Pb = 0.01 M; Cd = 0.006 M) and observed a decrease in Pb and an increase in Cd concentration after 4 weeks (Fortoul et al., 2005). Reductions in lung Pb were attributed to presence of Cd which decreased the dissolution of lung Pb, leading to less nebulization. However, increases in lung Cd were due to its affinity for thiol (–SH) groups, making it available in the lungs (Fortoul et al., 2005). Co-exposure of Pb, As and Mn to Wistar rats, increased the level of brain Pb, which was attributed to the presence of high affinity Pb binding proteins in the brain (Andrade et al., 2013).

Currently, studies show that toxic metals such as Pb, Hg, As and Cd can be deleterious to organisms even at very low concentrations (Huang et al., 2015). Our study therefore seeks to assess interactions associated with exposure of low dose toxic metal (Pb, Hg, As and Cd) mixtures, with toxic and essential metals in Institute of Cancer Research (ICR) mice. The doses of toxic metals used in this study, are the maximum permissible limits (MPLs) stipulated in the National Standard of The Republic of China for Municipal Water Standards (GB5749-2006). To our knowledge, this is the first study using such low concentrations to assess interactions among toxic and essential metals. The toxic heavy metals used were selected due to their public health importance, environmental abundance and common mechanisms shared in their toxicities (Wang and Fowler, 2008).

2. Materials and methods

2.1. Chemicals

Cadmium chloride, lead acetate, mercury chloride and sodium arsenite, which were of analytical grade, were purchased from Sinopharm Chemical Reagent Co. Ltd. Double distilled water was used in the preparation of stock solutions (1000 mg L^{-1}). Low concentrations of Pb (0.01 mg L^{-1}), Hg (0.001 mg L^{-1}), Cd (0.005 mg L^{-1}) and As (0.01 mg L^{-1}) were prepared through serial dilutions.

2.2. Animals and experimental design

Seven groups of three-week old ICR mice made up of twenty males (13.18 ± 1.80 g) and twenty females (11.98 ± 1.40 g) were purchased from the Comparative Medicine Center of the Yangzhou University in China. The upkeep of experimental animals was done in accordance with the method employed by Zhao et al. (2013b). The first group (control) was given distilled water and all animals were fed twice a day with basal diet (made up of carbohydrate 60%; protein 22%; fat 10% and others 8%). Six other groups made up of forty mice (20 males and 20 females kept separately) each, were exposed to metal mixtures made of binary, ternary and quaternary combinations (Table S1).

Test chemicals were administered to mice through free drinking for a period of 120 d. At the end of each month, 5 males and 5 females were selected randomly from each group for analysis. The selected mice were weighed, anaesthetized with sodium pentobarbital and blood samples collected through retro-orbital venous plexus. The brain, liver, and kidneys were removed, rinsed in cold saline water, weighed, and used for metal analyses.

2.3. Metal analysis

Toxic non-essential metals such as lead (Pb), mercury (Hg), cadmium (Cd), arsenic (As), and essential metals, such as calcium (Ca), magnesium (Mg), zinc (Zn), total iron (Fe), and copper (Cu) were determined after wet digestion according to the method described by Feist et al. (2008). Trace metal levels in brain, kidney and liver were determined using Vista-MPX Simultaneous ICP-OES (Varian, Inc. USA). Determinations were run in triplicates.

2.4. Heavy metal interaction using Combination Index (CI)

Interactions within metal mixtures in organs of mice were analyzed using the Combination Index (CI) according to Zhao et al. (2004). The CI, which is a numerical value, was calculated using (1) below;

$$\mathrm{CI} = \frac{C_{\mathrm{A},x}}{\mathrm{IC}_{x,\mathrm{A}}} + \frac{C_{\mathrm{B},x}}{\mathrm{IC}_{x,\mathrm{B}}} \quad (1)$$

where $C_{A,x}$ and $C_{B,x}$ are concentrations of metal toxicants A and B, used in combination to achieve an x% metal effect. $IC_{x,A}$ and $IC_{x,B}$ are concentrations for single metals to achieve the same effect. CI analysis provides qualitative information on the nature of metal interaction. A CI less than 1, greater than 1 or equal to 1 indicates synergism, antagonism, or additivity, respectively.

2.5. Statistical analysis

Statistical analyses were performed using SPSS 16.0 (SPSS Inc, Chicago, USA) statistical software. Results were expressed as mean ± SEM number of observations. Using one-way analysis of variance (ANOVA), the differences among groups were evaluated and considered statistically significant when $p < 0.05$, unless otherwise stated. Principal component analysis (PCA) and cluster analysis (CA) were conducted to discover the factors that could explain the correlation model between toxic and essential metals. Results of CA, were represented in a dendrogram, which depicts the levels of similarity between the different variables.

3. Results and discussion

Levels of individual metals in brain, liver, and kidneys of mice during 30- and 120-d studies are presented in Table S2.

3.1. Interaction with toxic metals

3.1.1. Brain of mice

The study assessed interactions between low dose metal mixtures with toxic metals in brain of mice. In 30-d, low dose Pb + Cd mixture increased brain Pb by 479%, whiles Pb + As and Pb + Cd increased it by 83.6% and 106%, respectively in 60 d. In 120-d study, Pb + Hg + As exposure, increased brain Pb significantly by 76.1%, however, Pb + As reduced brain Hg by 72.5% in 90-d. In 60 d, Pb + Hg, Pb + Cd and Pb + Hg + As increased brain As by 139%, 132% and 115%, respectively (Table 1).

Though the mechanism responsible for the elevation of brain Pb is not certain, deficiencies in essential metals such as Ca and Fe