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Interaction of four low dose toxic metals with essential metals in brain, liver and kidneys of mice on sub-chronic exposure

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ABSTRACT

This study reports on interactions between low dose toxic and essential metals. Low dose Pb (0.01 mg/L), Hg (0.001 mg/L), Cd (0.005 mg/L) and As (0.01 mg/L) were administered singly to four groups of 3-week old mice for 120 days. Pb exposure increased brain Mg and Cu by 55.5% and 266%, respectively. Increased brain Mg resulted from metabolic activity of brain to combat insults, whiles Cu overload was due to alteration and dysfunction of CTR1 and ATP7A molecules. Reduction of liver Ca by 56.0% and 31.6% (on exposure to As and Cd, respectively) resulted from inhibition of Ca-dependent ATPase in nuclei and endoplasmic reticulum through binding with thiol groups. Decreased kidney Mg, Ca and Fe was due to uptake of complexes of As and Cd with thiol groups from proximal tubular lumen. At considerably low doses, the study establishes that, toxic metals disturb the homeostasis of essential metals.

1. Introduction

Heavy metals are a group of environmental chemicals which are ubiquitous and non-biodegradable. Though adverse effects emanating from their exposure are widely known, their usage and concentrations in the environment is increasing (Wongsasuluk et al., 2014). Toxic effects of heavy metals on living systems are varied, ranging from immune system dysfunction (Jadhav et al., 2007a), embryogenesis (Fathallah et al., 2013), mortality to living organisms (Vellinger et al., 2012), mulfunction of neuronal systems (Hu et al., 2013; Rai et al., 2013), cancers of all sort (Feki-Tounsi et al., 2013), cytogenicity (Jadhav et al., 2006; Le et al., 2013; Varotto et al., 2013), and induction of oxidative stress (Jadhav et al., 2007b). Due to these

Abbreviations: Pb, lead; Hg, mercury; As, arsenic; Cd, cadmium; Ca, calcium; Mg, magnesium; Fe, iron; Zn, zinc; Cu, copper; CTR1, copper transporter protein; PCA, principal component analysis; CA, cluster analysis.

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adverse effects, studies on exposures to heavy metals are very significant.

On exposure to living systems various interactions occur which eventually affect organs and tissues of organisms depending on concentration of toxicants involved. Toxic metals tend to interact to generate reactive oxygen species (ROS) which enhances oxidative stress in the process and also interaction with macromolecules such as sulfhydryl, resulting in toxic effects to the organism (Wang and Fowler, 2008). In the environment most of these toxic metals are found mostly in very low concentrations. With this in mind, regulatory agencies set standards for drinking water taking into consideration levels of these toxic metals which are supposedly not harmful to human health. At these permissible limits it is assumed that no adverse effects would occur, however, after uptake, interactions with essential metals in the organism do happen. These interactions sometimes do occur through molecular mimicry of essential metals by the toxic ones, which eventually result in adverse effects to the organism.

There is currently little studies on the effect of exposure of low dose toxic metals on essential metals in an organism. This study seeks to assess the outcome of exposure of low dose toxic metals such as lead (Pb), mercury (Hg), cadmium (Cd) an arsenic (As) on essential metals. The concentrations of toxic metals used are the maximum permissible limits stipulated in the National Standard of The Republic of China for Municipal Water Standards (GB5749-2006). These are concentrations which, according to the regulatory agencies, are safe and will not induce adverse effects to water consumers. Essential metals that were considered in this study are magnesium (Mg), calcium (Ca), zinc (Zn), copper (Cu), iron (Fe). Given the widespread water quality challenges and associated health impacts faced by China, this study is timely. These toxic metals were selected due to their public health importance and the common mechanisms shared in their toxicities (*e.g.* generation of reactive oxygen species, interactions with sulfhydryl groups and interaction with essential metals) (Wang and Fowler, 2008).

2. Materials and methods

2.1. Chemicals

Analytical grade cadmium chloride, lead acetate, mercury chloride and sodium arsenite were purchased from Sinopharm Chemical Reagent Co. Ltd. Stock solutions of 1000 mg/L were prepared using double distilled water and low dose concentrations of 0.01 mg/L (Pb), 0.001 mg/L (Hg), 0.005 mg/L (Cd) and 0.01 mg/L (As) prepared through serial dilutions.

2.2. Animals

Five groups of 3-week old ICR mice made up of 20 males (13.18 ± 1.80 g) and 20 females (11.98 ± 1.40 g) were purchased from the Comparative Medicine Center of the Yangzhou University in China. Acclimatization of mice to their new environment was done for a period of three days before commencement of experiment. Mice were kept under controlled environmental conditions, which included a temperature of $22 \pm 2\,^{\circ}C$ and humidity of 55–60% during the experiment. Both male and female mice were kept separately in plastic cages padded with adsorbents at a 12-h light/dark cycle. Mice were fed with basal diet and given free access to drinking water. All experimental procedures conformed to *The Code of Ethics of the World Medical Association* for experiments involving humans (EC Directive 86/609/EEC for animal experiments). They were approved by the Jiangsu University Committee on Animal Care and Use.

2.3. Experimental design and exposure

Four groups made up of 40 mice (20 males and 20 females kept separately) each were exposed to low concentration heavy metals individually (Pb-0.01 mg/L; Hg-0.001 mg/L; Cd-0.005 mg/L; As-0.01 mg/L). The fifth group (control) was given distilled water and all animals were fed twice a day with basal diet. Test chemicals were administered to mice through drinking water for a period of 120 days. Mice were observed daily for adverse physical signs resulting from administration of test solutions. Signs such as deaths, deformation, rashes, hair loss were looked out for. At the end of each month 5 males and 5 females were selected randomly from each group for analysis. At the end of the experimental period (monthly), the selected mice were weighed, anaesthetized with sodium pentobarbital and blood samples collected through retro-orbital venous plexus. The brain, liver and kidneys of mice were removed, rinsed in cold saline water, weighed and used for metal analyses.

2.4. Metal analysis

Metals such as lead (Pb), mercury (Hg), Cadmium (Cd), arsenic (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 metals in brain, kidney and liver were determined using Vista-MPX Simultaneous ICP-OES (Varian, Inc. USA). Determinations were run in triplicates.

2.5. Statistical analysis

Statistical analyses were performed using SPSS 16.0. statistical software. Results were expressed as mean $\pm$ SD number of observations. Means were compared using one-way analysis of variance (ANOVA) and Duncan's multiple range test. Differences among groups were considered statistically significant when $p < 0.05$ unless otherwise stated. Principal component analysis (PCA) and cluster analysis (CA) were used to discover the factors that could explain the correlation model between essential metals. PCA was performed using the correlation matrix to identify possible associations and evaluate the extent of association between essential metals. Cluster analysis was used to identify homogenous groups of essential metals as a result of exposure to toxic metals. The Ward method was used and the Euclidean distance applied for the regrouping and identification of the distribution model of essential metals in mice. Results of CA were represented in a dendogram which depicts the levels of similarity between the