Differential Tissue Distribution of Copper in BALB/c Mice Following Exposure to Arsenic in Drinking Water

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Abstract: General population is exposed to elevated concentrations of arsenic (As), mainly via drinking water. As is known to be a potent human carcinogen and can disturb the distribution of essential metals, including copper (Cu). In this study, the tissue distribution and correlation between tissues for Cu was assessed in male BALB/c mice exposed to 0, 50, 500, or 5000 ppb As in their drinking water for 3 weeks. The Cu concentration in blood increased linearly with increasing As doses, whereas the Cu concentration in liver or kidney decreased linearly with respect to the dose applied. These data indicate that toxicity of As may be mediated by a disturbance in the distribution of essential metals, such as Cu.

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1. Introduction

Arsenic (As) is a ubiquitous metalloid found mainly in food, soil, and ground water. Because As in soil or rock is dissolved easily into surrounding water, inorganic As is frequently present at elevated concentrations in ground water (IARC, 2004; NRC, 2001; WHO, 2001). Consequently, more than one hundred million individuals who drink ground water are at risk of elevated As exposure via drinking water (Vahter, 2008).

Inorganic As in drinking water is known to be a potent human carcinogen, a causal factor of cancers in skin, urinary bladder, kidney, lung, and liver (IARC, 2004). In addition, chronic exposure to As through drinking water is associated with an increased risk of several non-cancer diseases that affect many organs, including the blood circulatory system, liver, and kidney (NRC, 2001; WHO, 2001).

The mechanisms underlying As toxicity and/or carcinogenicity remain unclear. However, one study has demonstrated that the relationship between toxic metal exposure and tissue concentrations of essential metals over prolonged exposures could be an important factor for chronic toxicities, which were mediated by a disturbance in the distribution of essential metals, such as copper (Cu), rather than the exposed metal alone (Blazovics et al., 2004).

Cu is an essential nutrient required for the activities of important enzymes such as cytochrome c oxidase and lysyl oxidase, whereas the reactive nature of Cu ions can also cause cellular damage as a result of free radical generation (Pena et al., 1999). Therefore, tissue Cu concentrations and distribution in the body are strictly regulated to ensure an adequate supply of Cu to cuproenzymes and the removal of excess Cu. The importance of Cu homeostasis is demonstrated by the severity of genetic disorders that affect Cu metabolism, such as Menkes disease and Wilson disease (Ke et al., 2008).

To explore the mechanism of As toxicity concerning Cu homeostasis, we investigated whether subacute exposure to As in drinking water may change the concentrations of Cu in blood, liver and kidney, as well as the correlation of Cu concentrations among these organs

2. Material and Methods

2.1. Animals. BALB/c mice (n = 40, 7-week-old males weighing 24.3 ± 1.0 g) were divided into four groups: saline-treated control and 50, 500, and 5000 ppb As-treated groups. Mice (n = 10 per group) groups received 0, 50, 500 or 5000 ppb As in the form of $NaAsO_2$ in their drinking water for 3 weeks. Control animals were given distilled deionized water only. Water consumption was measured throughout the study period, and the mice were weighed weekly. Mice and $NaAsO_2$ were purchased from Samtako Laboratories (Osan, Korea) and Wako Chemical Co. (Osaka, Japan), respectively. Other reagents were of the highest quality available and were obtained from commercial sources. Experiments were approved by the Institutional Animal Care and Use Committee of Keimyung University, Korea. Experiments were conducted according to the NIH guidelines for the care and use of laboratory animals. Animals were housed in a specific pathogen-free (SPF) facility,

with free access to food and water. At the end of the treatment regimen, mice were sacrificed by CO_2 asphyxiation, and blood, liver, and kidney were harvested immediately. Tissue samples were placed immediately into cryovials and stored at –70°C until use.

2.2. Analytical methods. Once ready for analysis, the tissue samples were thawed, and about 0.5 ml of blood or 50 mg of dry tissue was digested with 10 ml HNO_3 (70%) in a microwave digestion system. The Cu content was determined by inductively coupled plasma-mass spectrometry (Elan 900; Perkin-Elmer Co., Norwalk, USA). The analytical performance is assessed periodically through participation in the Korea Food and Drug Administration's proficiency testing program for trace elements in whole blood and tissue. Internal quality control (IQC) materials covering the expected range of concentrations were analyzed at the beginning and end of each batch of specimens and throughout each analytical run.

2.3. Statistical analysis. The Cu concentrations in the tissues of As-treated animals were compared to those of the controls using a one-way analysis of variance followed by a *post hoc* Duncan test. Statistical significance was defined as $P < 0.05$ or $P < 0.01$. Mean values were calculated, along with the standard deviation (SD) or standard error (SE) for each mean value. All statistical analyses were conducted using SAS 9.2 software (SAS Institute Inc., Cary, USA).

3. Results

No signs of overt toxicity were observed in the mice throughout the duration of the study.

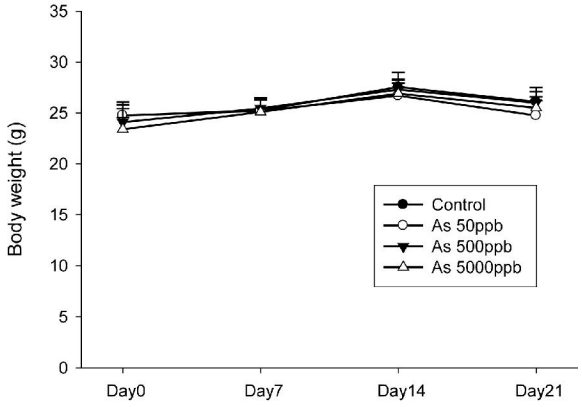

Figure 1. Average weekly body weight during 3 weeks of exposure to As in drinking water. No significant differences were observed in body weight gain among exposure groups. Values are the mean ± SD in each group (n = 10).

Body weights were slightly lower at the time of terminal necropsy due to overnight fasting, body weight gain was not significantly affected by As exposure (Figure 1). The water intake did not differ significantly among the treatment groups. The calculated average daily As intakes determined weekly and averaged over the entire study period were 10.5 ± 0.9, 89.2 ± 13.9, and 1039.4 ± 171.6 mg sodium arsenite/kg body weight/day for the 50, 500, and 5000 ppb groups, respectively.

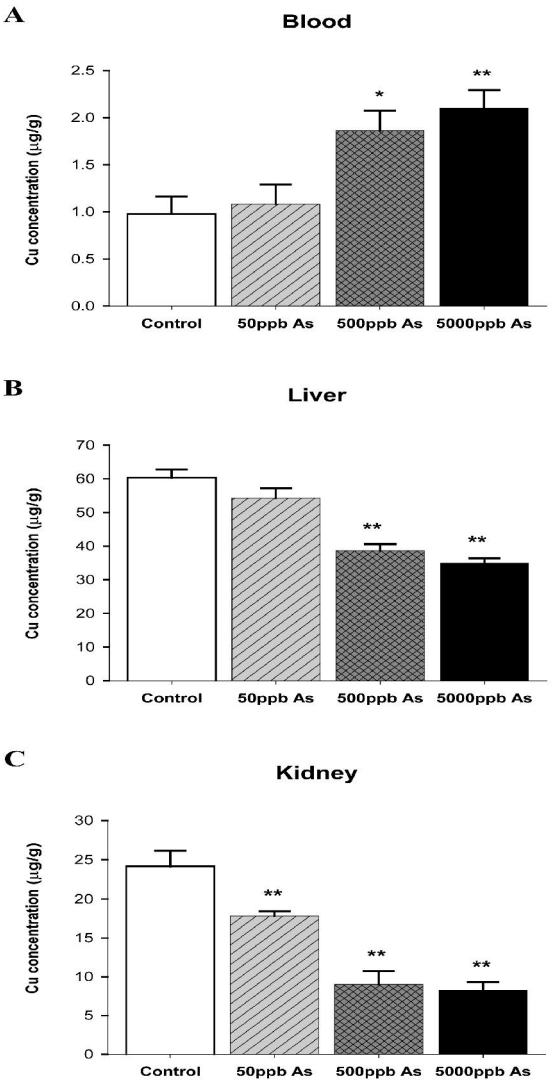

Figure 2. Concentration of Cu in blood (A), liver (B), and kidney (C) after 3 weeks of exposure to 50, 500, and 5000 ppb As in drinking water. Values are the mean ± SE in each group (n = 10). *$P < 0.05$, **$P < 0.01$, compared to the control group.

Concentration data for the Cu detected in whole blood, liver, and kidney at the end of 3 weeks of drinking water exposure to As were above the detection limits and are presented in Figure 2. Cu concentrations were the lowest in blood compared to liver or kidney (Figure 2A). In blood, the Cu concentration increased dose-dependently with increasing As treatment concentration and was significantly elevated in the 500 ppb ($P < 0.05$) and 5000 ppb ($P < 0.01$) groups compared to the control. Contrary to the Cu level in blood, the Cu levels in liver and kidney indicated a dose-dependent decrease with increasing As treatment concentrations. Especially, Cu levels in liver of the 500 and 5000 ppb As-treated groups, and those in kidney of the 50, 500, and 5000 ppb As-treated groups showed statistically significant decreases compared to the control ($P < 0.01$).

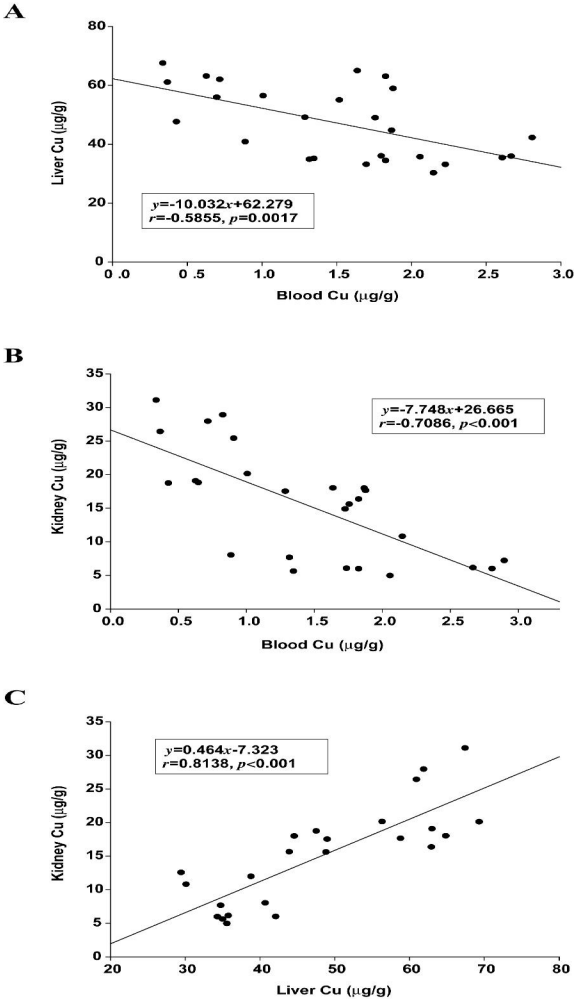

Figure 3. Correlations between liver Cu and blood Cu (A), between kidney Cu and blood Cu (B), and between kidney Cu and liver Cu (C). Data were analyzed by linear correlation.

A linear regression analysis was used to examine whether the Cu concentrations were correlated among blood, liver, and kidney. When blood concentrations of Cu were plotted against liver or kidney concentrations of Cu, significant inverse correlations were observed between blood and liver (correlation coefficient (r) = –0.5855, P = 0.0017) and between blood and kidney (r = –0.7086, $P < 0.001$) (Figs. 3A and 3B). However, the Cu concentrations between liver and kidney exhibited a significant positive association (r = 0.8138, $P < 0.001$; Figure 3C).

4. Discussions

This study reports Cu accumulation in blood, liver, and kidney across a wide dose range under conditions of subchronic exposure to As in drinking water. Our results clearly demonstrate that the distribution and accumulation of Cu is both tissue-specific and dose-dependent, and it is correlated among tissues.

Physiological balance among trace elements is essential for human health and is controlled by metal-binding proteins, which comprise one third of all mammalian proteins (Gailer, 2007). Metallothioneins (MTs) are one of the major inducible metal-binding proteins involved in homeostasis and the detoxification of metals (Bremner and Beattie, 1990). MT binds preferentially to cadmium and zinc, but it also has the capacity to bind As and Cu (Hidalgo et al., 2001; Nordberg and Nordberg, 2000; Toyama et al., 2002). Moreover, As is known to be an inducer of MTs (Kreppel et al., 1993; Goering and Klaassen, 1983). Consequently, excess levels of As can compete or interfere with essential elements, including Cu (Goyer, 1997).

These results suggest that As-induced toxicity may be induced in part by the disturbance of Cu homeostasis. However, further studies are needed to fully address the effects of As on the tissue distribution of Cu and whether changes in Cu concentration in tissues are a primary or secondary effect of As treatment.

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