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Cardiovascular responses to lead are biphasic, while methylmercury, but not inorganic mercury, monotonically increases blood pressure in rats

Tanja M. Wildemann [a,b], Naghmeh Mirhosseini [c], Steven D. Siciliano [a,b], Lynn P. Weber [a,c,*]

[a] Toxicology Graduate Program, University of Saskatchewan, 44 Campus Drive, Saskatoon, SK S7N 5B3, Canada
[b] Department of Soil Science, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK S7N 5A8, Canada
[c] Department of Veterinary Biomedical Sciences, University of Saskatchewan, 52 Campus Drive, Saskatoon, SK S7N 5B4, Canada

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ABSTRACT

Cardiovascular diseases, such as heart attack and stroke, are the major cause of death worldwide. It is well known that a high number of environmental and physiological risk factors contribute to the development of cardiovascular diseases. Although risk factors are additive, increased blood pressure (hypertension) is the greatest risk factor. Over the last two decades, a growing number of epidemiological studies associate environmental exposure to lead or mercury species with hypertension. However, cardiovascular effects beyond blood pressure are rarely studied and thresholds for effect are not yet clear. To explore effects of lead or mercury species on the cardiovascular system, normal male Wistar rats were exposed to a range of doses of lead, inorganic mercury or methylmercury through the drinking water for four weeks. High-resolution ultrasound was used to measure heart and vascular function (carotid artery blood flow) at baseline and at the end of the exposure, while blood pressure was measured directly in the femoral artery at the end of the 4-week exposure. After 4 weeks, blood pressure responses to lead were biphasic. Low lead levels decreased blood pressure, dilated the carotid artery and increased cardiac output. At higher lead doses, rats had increased blood pressure. In contrast, methylmercury-exposed rats had increased blood pressure at all doses despite dilated carotid arteries. Inorganic mercury did not show any significant cardiovascular effects. Based on the current study, the benchmark dose level 10% ($BMDL_{10}$s) for systolic blood pressure for lead, inorganic mercury and methylmercury are 1.1, 1.3 and 1.0 μg/kg-bw/d, respectively. However, similar total mercury blood levels attributed to inorganic mercury or methylmercury produced strikingly different results with inorganic mercury having no observable effect on the cardiovascular system but methylmercury increasing systolic and pulse pressures. Therefore, adverse cardiovascular effects cannot be predicted by total blood mercury level alone and the mercury species of exposure must be taken into account.

1. Introduction

Heavy metals, such as lead (Pb) and mercury (Hg), are mobilized in the environment mainly through anthropogenic sources, such as mining and industrial activities, rendering them ubiquitously present in the environment (ATSDR, 2007; Health Canada, 2013; EFSA, 2012a). The principal exposure route for lead is oral through food and drinking water (Health Canada, 2013; ATSDR, 1999; EFSA, 2012a). The absorption of lead depends on a variety of factors, such as age or the nutritional status of the person. In the body, lead is bound to the erythrocytes and more than 90% of the total body burden in adults can be found in the bones (ATSDR, 2007). Two mercury species are relevant for human exposure, namely elemental mercury (Hg^0) and methylmercury (MeHg). While exposure to elemental mercury primarily arises through inhalation from dental amalgam fillings (Richardson, 2014), methylmercury accumulates in predatory fish and seafood, which leads to dietary exposure in humans (Clarkson, 2002). Elemental mercury is vaporous and hence very mobile. It

* Corresponding author at: Department of Veterinary Biomedical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, 52 Campus Drive, Saskatoon, SK S7N 5B4, Canada. Tel.: +1 306 966 8734.
E-mail addresses: tanja.wildemann@usask.ca (T.M. Wildemann), n.mir@usask.ca (N. Mirhosseini), steven.siciliano@usask.ca (S.D. Siciliano), lynn.weber@usask.ca (L.P. Weber).

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can easily cross the blood brain barrier, but is also quickly oxidized to inorganic mercury in the blood and other tissues (Clarkson et al., 2007), leading to deposition of inorganic mercury in target organs such as liver or kidneys. Methylmercury is readily absorbed in the gut and distributes in all tissues, including the brain. It can be converted in the body to inorganic mercury or methylmercury–cysteine which can mimic the amino acid methionine (Clarkson et al., 2007; Ballatori, 2002). Thus, human environmental mercury exposure can be modeled with inorganic mercury and methylmercury.

Although both lead and mercury are considered to have neurological effects as their primary toxicity, a growing body of epidemiological research associates lead exposure (mostly blood lead levels) with adverse cardiovascular health. Data from population-based studies, including the National Health and Nutrition Examination Survey (NHANES) or the Normative Aging Study, show clear associations between low blood lead levels ($\leq$10 μg/dl) and hypertension (Scinicariello et al., 2011; Cheng et al., 2001; Nash et al., 2003; Glenn et al., 2003). Only a few studies using the NHANES database examined additional cardiovascular end-points beyond hypertension. Specifically, positive associations were shown between blood lead levels ($\geq$2 μg/dL) and increased mortality due to myocardial infarction and stroke (Menke et al., 2006; Schober et al., 2006), increased risk of peripheral arterial disease (Navas-Acien et al., 2004) or abnormalities in electrocardiogram (ECG) data consistent with left ventricular hypertrophy (Schwartz, 1991). A few rat studies support a cause-effect relationship between chronic lead exposure and hypertension (Nowack et al., 1993; Fiorim et al., 2011; Ding et al., 1998). Furthermore, Skoczynska et al. (2013) exposed rats to lead through the drinking water and then measured heart function with cardiac magnetic resonance imaging (MRI). This study showed that the cardiac ejection fraction and fractional area change in the lead-exposed rats were significantly reduced in comparison to the control rats indicating impaired cardiac function. There is evidence for a causal relationship between blood lead levels and hypertension as well as additional cardiac effects but thresholds for adverse effects remain unclear.

The connection between mercury exposure and cardiovascular health is much less clear. In human studies, the most common biomarker of exposure is total mercury levels in blood which is generally thought to indicate recent exposure to inorganic and organic mercury (Health Canada, 2010). Another biomarker of exposure is mercury levels in hair samples which are generally thought to relate to methylmercury exposure (Li et al., 2008). Some epidemiological studies (Bautista et al., 2009; Pedersen et al., 2005; Valera et al., 2009) found a positive association between blood or hair mercury levels and an increased risk for hypertension and pulse pressure. However, other studies in humans (Johansson et al., 2002; Mozaffarian et al., 2011, 2012; Vupputuri et al., 2005; Valera et al., 2011a; Park et al., 2013) did not find a link between blood mercury levels and blood pressure or cardiovascular diseases (Mozaffarian et al., 2011). Higher mercury levels in hair or blood samples were associated with reduced heart rate variability (a known risk factor for cardiovascular death) in Koreans (Lim et al., 2010), Nunavik Inuits (Valera et al., 2008) and Canadian Cree adults (Valera et al., 2011a). Furthermore, Salonen et al. (Salonen et al., 2000) found a positive association between hair mercury levels and carotid atherosclerosis in a Finnish cohort study. Finally, dentists and dental staff are occupationally exposed to mercury vapor, which is quickly metabolized to inorganic mercury, through the handling of dental amalgam. Using pharmacy utilization data, Duplinsky and Cicchetti (2012) showed that dental professionals at the age of 45 and above have a higher prescription rate for cardiovascular medication than the general population, further supporting the link between higher mercury exposure and increased risk for cardiovascular disease. In summary, while human studies show an association between mercury exposure and cardiovascular health, the specific mercury species responsible and the thresholds for cardiovascular effect remain unclear.

Animal studies have also suggested a link between mercury and adverse cardiovascular effects. In rat studies, although inorganic mercury (50 μg $HgCl_2$/ml in the drinking water for 320 days or 4.6 μg/kg injected loading dose plus daily 0.07 μg/kg/day i.m.) failed to significantly affect blood pressure (Carmignani et al., 1983; Blanco-Rivero et al., 2011; Furieri et al., 2011a), other studies using the same doses have reported increased vasoconstriction in isolated arteries (Golpon et al., 2003; Blanco-Rivero et al., 2011; Furieri et al., 2011b) or reduced cardiac contractility in isolated rat hearts (Souza de Assis et al., 2003; Furieri et al., 2011a). In contrast, methylmercury exposure in rats (0.5 mg MeHg/kg-bw oral gavage for 23–28 days or a 10-fold higher dose) led to latent increases in systolic blood pressure either at 42 days or immediately after exposure, respectively (Wakita, 1987). In another study, rats gavaged with 100 μg/kg-bw/d methylmercury for 100 days also had significantly increased systolic blood pressure after four weeks (Grotto et al., 2009). Therefore, animal studies are highly suggestive of a causative association between methylmercury exposure and adverse cardiovascular effects, while the relationship between inorganic mercury and cardiovascular effects are unclear. However, thresholds for adverse cardiovascular effects for both mercury species require clarification.

We hypothesized that exposures to lead, inorganic mercury or methylmercury will adversely affect cardiovascular function in rats. In order to investigate adverse effects of lead versus differing mercury species on the cardiovascular system, as well as the thresholds for these effects, we examined effects of a broad range of metal doses in normal male Wistar rats. In order to generate data that could be used to assess risk to humans through oral exposure, rats were exposed to lead acetate, mercury chloride or monomethylmercury chloride for 28 days via the drinking water. Cardiovascular end-points evaluated included cardiac and vascular function, assessed by high-resolution ultrasound in B-mode and power Doppler mode, respectively; direct (intravascular) blood pressure was measured in the femoral artery, and cardiac electrical activity through electrocardiography (ECG) analyses at the end of the 4-week exposure.

2. Materials and methods

2.1. Animals

Male Wistar rats (250–300 g) were purchased from Charles River Laboratories, Canada and acclimatized for one week before the start of the experiment. The animals were housed singly at the Western College of Veterinary Medicine at the University of Saskatchewan (Saskatoon, SK, Canada). They were housed at 22 °C under a 12:12 h-light dark cycle with free access to standard rat chow. All experiments were approved by the University of Saskatchewan's Animal Research Ethics Board and carried out according to the guidelines of the Canadian Council on Animal Care (CCAC).

For the duration of four weeks, rats were exposed (n = 5–6) to either lead acetate (Pb(II)), mercury chloride (Hg(II)) or monomethylmercury chloride (MeHg(I)) through *ad libitum* drinking water (tap water with 0.2% nitric acid). Rats were exposed to broad ranges of lead acetate (4, 7, 14, 29, 57, 357, 1607, 45,000 μg/kg-bw/d) or mercury chloride (7, 14, 29, 57, 357, 2000, 4000, 8000 μg/kg-bw/d) or mono-methylmercury chloride (4, 7, 14, 29, 57, 357, 1607 μg/kg-bw/d) based on published studies and LD_{50} (Malvezzi et al., 2001; Carmignani et al., 1983, 1992; Grotto et al., 2009; Jin