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The mechanisms associated with the development of hypertension after exposure to lead, mercury species or their mixtures differs with the metal and the mixture ratio

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ABSTRACT

Hypertension is considered to be the most important risk factor for the development of cardiovascular diseases. Beside life-style risk factors, exposure to lead and mercury species are increasingly discussed as potential risk factors. Although there are a few previous studies, the underlying mechanism by which exposure to lead and mercury disturb blood pressure regulation is not currently understood. Potential mechanisms are oxidative stress production, kidney damage and activation of the renin–angiotensin system (RAS), all of which can interact to cause dysregulation of blood pressure. Male rats (Wistar) were exposed to lead, inorganic mercury, methylmercury or two mixtures of all three metals for four weeks through the drinking water. The two mixture ratios were based on ratios of known reference values or environmental exposure from the literature. To investigate the potential mechanism of actions, blood pressure was measured after four weeks and compared to plasma nitrotyrosine or reduced/oxidized glutathione levels in liver as markers for oxidative stress. Plasma renin and angiotensin II levels were used as markers for RAS activation. Finally, kidney function and injury were assessed via urinary and plasma creatinine levels, creatinine clearance and urinary kidney-injury molecule (KIM-1). While exposure to lead by itself increased oxidative stress and kidney damage along with blood pressure, inorganic mercury did not affect blood pressure or any end-point examined. Conversely, methylmercury instead increased RAS activation along with blood pressure. Surprisingly, when administered as mixtures, lead no longer increased oxidative stress or altered kidney function. Moreover, the mixture based on an environmental ratio no longer had an effect on blood pressure, while the reference value ratio still retained an increase in blood pressure. Based on our results, the prominent mechanism of action associated with the development of hypertension seems to be oxidative stress and kidney damage for lead, while increased RAS activation links methylmercury to hypertension, but these mechanisms along with hypertension disappear when metals are present in some mixtures.

1. Introduction

Cardiovascular disease in humans such as heart attacks and stroke, present a worldwide health problem because it is responsible for the majority of deaths and hypertension is considered to be the most important risk factor (Mendis et al., 2011). While life-style risk factors are known to increase blood pressure, increasingly environmental pollutants such as heavy metals are viewed as potential risk factors contributing to hypertension (Nawrot et al., 2002; Navas-Acien et al., 2007; Glenn et al., 2003). The human population is exposed chronically to low levels of lead and mercury through the environment (Nawrot et al., 2002; Pedersen et al., 2005; Bautista et al., 2009; Valera et al., 2009; Roman et al., 2011; Park et al., 2013; Peters et al., 2012). Based on the scientific evidence from human epidemiology studies, lead (Pb(II)) is now considered to have a causal relationship with hypertension (Nawrot et al., 2002; Navas-Acien et al., 2007; Glenn et al., 2003). The relationship between mercury exposure and hypertension is less clear. A positive association between mercury exposure and hypertension was found in some

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human studies (Bautista et al., 2009; Valera et al., 2009; Pedersen et al., 2005), while a negative association was found in another set of epidemiological studies (Vupputuri et al., 2005; Johansson et al., 2002; Mozaffarian et al., 2011, 2012; Valera et al., 2011; Park et al., 2013).

Despite an increasing number of mechanistic studies, the underlying mechanism(s) by which exposures to Pb(II), inorganic mercury (Hg(II)), organic mercury (MeHg(I)) or their mixtures influence blood pressure is not understood. One leading hypothesis for mechanism of action of metals is oxidative stress. Exposure to either Pb(II) or Hg has been reported to cause increased production of reactive oxygen species (ROS) in humans (Ercal et al., 2001), depletion of antioxidant defenses such as reduced glutathione or both in vitro (Saeidnia and Abdollahi, 2013). In addition, studies have shown that Pb(II) exposure increased the production of ROS in rats such as superoxide anion radical and this secondarily decreased the availability of nitric oxide (NO) leading to the production of the highly toxic peroxynitrite (Farmand et al., 2005; Ding et al., 2001; Vaziri et al., 2003; Vaziri, 2008). At the same time, Pb(II) exposure is associated with reduced available glutathione (GSH) in humans (Kasperczyk et al., 2004). Similarly, Hg(II) and MeHg(I) have also been reported to decrease the availability of NO through increased ROS production in humans and rats (Wiggers et al., 2008a; de Marco et al., 2009; Lemos et al., 2012). In particular MeHg(I) has a high chemical affinity for the sulfhydryl groups of glutathione, resulting in inactivated glutathione and depleted antioxidant defenses (Ballatori, 2002). The well-known consequences of disturbances in ROS production, depleted antioxidants and NO inactivation are numerous, but include endothelial dysfunction, vasoconstriction and hypertension in a variety of species (Li and Foerstermann, 2000).

The renin–angiotensin-system (RAS) is directly involved in blood pressure regulation through the control of peripheral vascular resistance (Peach, 1977). A study on acute exposure to lead in rats (Simoes et al., 2011) investigated the mechanism underlying the early stages of hypertension. This study showed that acute lead exposure increases the activity of the angiotensin-converting enzyme (ACE), which catalyzes the conversion from angiotensin I to angiotensin II. Comparable results were observed when rats were chronically exposed to lead (Carmignani et al., 1999; Sharifi et al., 2004). However, the activation of the RAS system by lead exposure appears to depend on a number of factors, such as length of exposure or age in both humans and rats (Vander, 1988). Similar results were found when rats were exposed to inorganic mercury. While renin was decreased (Carmignani et al., 1992), the activity of angiotensin converting enzyme (ACE) was augmented (Carmignani et al., 1992; Wiggers et al., 2008b). To our knowledge, no previous studies have examined the effect of methylmercury exposure on RAS activity.

In addition to the central nervous system, the kidneys play a crucial role in blood pressure regulation (Wadei and Textor, 2012). Therefore, impaired kidney function due to exposure to Pb(II) and Hg is a second mechanism that may be important for the development of hypertension. Analysis of data from the Third National Health and Nutrition Examination Survey (NHANES) (Muntner et al., 2003) and the Normative Aging Study (Tsaih et al., 2004) showed positive associations between human Pb body burdens and increased serum creatinine concentrations, a biomarker for impaired kidney function (Vlasakova et al., 2014). The kidneys are the target organ for the accumulation of Hg(II), making Hg(II) particularly nephrotoxic (Zalups, 2000). The proximal tubule of the nephron (Zalups, 2000; Massanyi et al., 2007) is the main target of Hg(II) in rats, resulting in increased serum creatinine levels in this species (Shi et al., 2011).

A previous study in our lab (Wildemann et al., 2015a) showed that exposures to Pb(II) or MeHg(I), but not Hg(II), each as single metals, increased blood pressure in rats. Paradoxically, in a follow-up study (Wildemann et al., 2015b), we found that some mixtures of these three metals had no effect on blood pressure, depending on the relative ratios. These paradoxical results were puzzling since metal blood levels were similar or higher after mixture exposures compared to the metals alone (Wildemann et al., 2015b), indicating that altered bioaccumulation was not the mechanism responsible for loss of blood pressure effect of metal mixtures. Based on our previous studies, if toxicokinetics do not explain altered blood pressure responses in single metal versus mixture exposures, then a change in mechanisms of effect may instead provide an explanation. Therefore, we hypothesized that mechanisms of action after oral exposure to Pb(II), Hg(II), MeHg(I) might differ when exposures were to metals alone versus in mixtures and that this might be linked to blood pressure changes. In order to investigate this hypothesis, we exposed male Wistar rats for 4 weeks to Pb(II), Hg(II), MeHg(I), alone or in combination, then assessed blood pressure, oxidative stress (plasma nitrotyrosine and liver oxidized/reduced GSH), RAS activation (plasma renin and angiotensin II) and kidney function/damage (urinary and plasma creatinine, creatinine clearance and KIM-1). The patterns of change in these metal mechanisms of action were then compared to alterations in blood pressure after four weeks of exposure in male rats.

2. Materials and methods

2.1. Animals and exposures

Chemicals were obtained from Sigma–Aldrich (Oakville, ON, Canada). The Animal Research Ethics Board at the University of Saskatchewan approved all procedures in this experiment according to the guidelines of the Canadian Council on Animal Care (CCAC). Male rats (Wistar strain, 250–300 g, 2–3 months of age) were obtained from Charles River Laboratories, Senneville, QC, Canada and were housed in single cages, at 22 °C room temperature and a 12:12H-light dark cycle at the Western College of Veterinary Medicine at the University of Saskatchewan (Saskatoon, SK, Canada). We have published a previous study from this group (Wildemann et al., 2015b) using the same cohort of rats as the current study, but the current study used a separate sub-sample than this previously published study. The animals were randomly assigned to the different treatment groups, acclimatized for one week and had access to standard rat chow ad libitum. For the duration of four weeks, rats ($n = 5$–6/group) received either lead acetate (Pb(II)), mercury chloride (Hg(II)), mono-methylmercury chloride (MeHg(I)) or a mixture of all three metals through the drinking water (tap water with 0.2% nitric acid) as described in Wildemann et al. (2015b). Briefly, rats exposed to Pb(II) received either 1607 or 45,000 μg Pb(II)/kg-bw/d, to Hg(II) either 357 or 4000 μg Hg(II)/kg-bw/d and to MeHg(I) either 7 or 357 μg MeHg (I)/kg-bw/d via the drinking water.

A fixed ray design was used to examine effects of mixtures of these three metals in the current and previous experiment (Wildemann et al., 2015b), with one ray based on reference values (R) used by several regulatory agencies and one based on commonly reported environmental levels of these metals (E). Briefly, for reference values, we used a 1% bench mark dose limit or $BMDL_{01}$ value for Pb(II) and systolic blood pressure as an end-point reported by the European Food Safety Authority (EFSA, 2012b) of 1.5 μg/kg-bw/d. The Joint FAO/WHO Expert Committee on Food Additives (JECFA; FAO/WHO, 2004) set a tolerable weekly intake (TWI) of 1.6 μg/kg-bw for methylmercury which would equal 0.23 μg/kg-bw per day. JECFA (FAO/WHO, 2011, 2004) also established a TWI of 4 μg/kg-bw for inorganic mercury corresponding to 0.57 μg/kg-bw per day. Both TWIs were confirmed by