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Toxic effects of mercury, lead and gadolinium on vascular reactivity

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Abstract

Heavy metals have been used in a wide variety of human activities that have significantly increased both professional and environmental exposure. Unfortunately, disasters have highlighted the toxic effects of metals on different organs and systems. Over the last 50 years, the adverse effects of chronic lead, mercury and gadolinium exposure have been underscored. Mercury and lead induce hypertension in humans and animals, affecting endothelial function in addition to their other effects. Increased cardiovascular risk after exposure to metals has been reported, but the underlying mechanisms, mainly for short periods of time and at low concentrations, have not been well explored. The presence of other metals such as gadolinium has raised concerns about contrast-induced nephropathy and, interestingly, despite this negative action, gadolinium has not been defined as a toxic agent. The main actions of these metals, demonstrated in animal and human studies, are an increase of free radical production and oxidative stress and stimulation of angiotensin I-converting enzyme activity, among others. Increased vascular reactivity, highlighted in the present review, resulting from these actions might be an important mechanism underlying increased cardiovascular risk. Finally, the results described in this review suggest that mercury, lead and gadolinium, even at low doses or concentrations, affect vascular reactivity. Acting via the endothelium, by continuous exposure followed by their absorption, they can increase the production of free radicals and of angiotensin II, representing a hazard for cardiovascular function. In addition, the actual reference values, considered to pose no risk, need to be reduced.

Key words: Heavy metal toxicity; Vascular reactivity; Mercury; Lead; Gadolinium

Historical background

The use of heavy metals is intimately connected to human history. Men discovered metals as important materials for making tools and implements as early as during prehistorical times. However, recorded information about metals does not simply describe their benefits. The contacts of human beings with metal compounds that have no physiological activity, even at low concentrations, are correlated to high levels of toxicity (1). Nowadays several industrial activities and agriculture generate a wide variety of chemical species containing lead, mercury and cadmium, which are

in general associated with environmental pollution because of their toxicity and bioaccumulation (1). Metals are also used as ingredients in several compounds for treatments, conservation of vaccines (2) or contrast medium, as is the case for gadolinium (3).

These metals usually induce free radical production and mainly interact with sulfhydryl groups in proteins, altering their activities. The biochemical basis for these effects is often attributed to the inhibition of several ATPases, including Na,K-ATPase (NKA) and Ca-ATPase (4-8), angiotensin I-converting enzyme (ACE), and to the production of oxidative damage (free radicals by activation of NADPH oxidase).

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Damage resulting from acute or chronic exposure to toxic metals has been mainly described for the nervous system and kidneys (4,8-10). However, increasing knowledge about vascular function including the endothelial-derived mechanisms that regulate vascular tone and the role of enzymes, such as NKA and ACE, and free radicals, has mainly been obtained about metals with vascular function that promote endothelial dysfunction, hypertension and vascular diseases. Recently, the fact that the endothelium is affected by low doses or concentrations of heavy metals has highlighted the importance and the need of improved knowledge of the mechanisms by which these metals promote the development of cardiovascular diseases.

In this review, we will focus on the vascular actions of mercury, lead and gadolinium, heavy metals that we have been studying in our laboratory.

Mercury

Mercury is worthy of note in this context because of its high toxicity and great mobility in ecosystems. Mercury is known to exert its effects by combining with SH groups (4,11), which are essential for the normal function of several proteins that constitute enzymes, ion channels or receptors (11,12). These effects include inhibition of ATP hydrolysis and NKA and Ca^{2+} -ATPase activity (5,7).

The US Environmental Protection Agency recommends the reference blood concentration of mercury to be below that considered without adverse effects, which is 5.8 ng/mL (13). However, in unexposed populations taken as controls, blood mercury concentration was reported to be 2.73 ng/mL in New York City adults and 1 ng/mL in China (14,15). For many years mercury was used in diuretics or as an ingredient of antiseptics (4). However, after mercurials were replaced with more specific treatments, signs of mercury intoxication have become rare (10). However, in the past decade, mercury intoxication and poisoning attained high levels (9,16,17) as a result of environmental pollution. The Minamata Bay episode resulting from industrial plastic disposal, the ingestion of contaminated wheat seeds in Guatemala, Iraq and Pakistan, and contamination from metallic mercury used in gold mining in Brazil are examples of this problem (1,17). Human toxicological data about methylmercury poisoning show that symptoms of toxic effects appear at a concentration of mercury in blood of 0.1 $\mu\text{g/mL}$ and death occurs at concentrations above 3 $\mu\text{g/mL}$ (9). It has also been reported that professional exposure to mercury vapor and the release of mercury from or during the removal of amalgam dental fillings increases the blood and plasma concentration of the metal (18-20). After exposure to mercury vapor, blood concentrations attain 18 nM (20) and, after exposure to dental amalgam fillings and their removal, plasma concentration attains 5 nM (19). Another important mechanism of exposure is fish consumption. Mercury attains 5.65 ng/mL among regular fish consumers (13,14) and levels

of 7 to 10 ng/mL among exposed workers (10,15).

Recently, more attention has been paid to the toxic effects of mercury on the cardiovascular system and their association with hypertension, carotid atherosclerosis, myocardial infarction, and coronary heart disease (21-23). In the cardiovascular system, acute mercury exposure promotes reduction of myocardial force development (24) and inhibition of myosin ATPase activity (24). Chronic exposure to this metal increases vascular resistance (25,26) and induces hypertension (21).

Functional integrity of the endothelium is crucial for the maintenance of blood flow and antithrombotic capacity because the endothelium releases humoral factors that control relaxation and contraction, thrombogenesis and fibrinogenesis and platelet activation and inhibition. Previous studies have demonstrated that mercury decreases the production of nitric oxide (NO) and alters the expression of NO synthase (NOS) (27). HgCl_2 at concentrations of 0.5 to 10 μM produces vasoconstriction, reduces the endothelial vasodilator response and stimulates a cyclooxygenase (COX)-derived vasoconstrictor (28). In addition, several studies have shown that mercury induces oxidative stress with subsequent oxidative damage to several organs or systems (23). Glutathione (GSH) depletion by mercury may be a trigger for the production of reactive oxygen species (ROS) that induce lipid, protein and DNA oxidation (23). Vascular endothelium is highly sensitive to oxidative stress and this is the main cause of endothelial dysfunction observed in cardiovascular diseases such as hypertension and atherosclerosis (22,29). Most mercury toxicity studies are performed with high (micromolar) concentrations of mercury and under acute exposure. Then, given the idea that relatively high blood levels are more frequently used to indicate mercury as an environmental risk factor for cardiovascular diseases, we recently developed a methodology involving controlled chronic mercury administration that attains a concentration of 8 ng/mL (~ 29 nM) in blood (25) to better understand the endothelial modulation of vascular responses.

Lead

Lead is abundant in the earth's crust, with average concentrations between 10 and 20 mg/kg in the soil. The greatest natural sources are volcanic activity and geochemical weathering. It is estimated that the natural emissions of lead are about 19,000 tons. However, anthropogenic processes such as painting, addition of lead to petrol, battery manufacturing, etc., are important processes responsible for the environmental presence of lead (30). Once lead particles go into the air they are removed by deposition, transferred to the surface and rapidly distributed in water or sediments according to the pH of dissolved salts (30). Therefore, lead has become one of the most common environmental contaminants resulting from external sources.

This metal is recognized as a common environmental and occupational health hazard (31) because of numerous industrial activities that favor its wide distribution. Human beings usually have lead in their organism (8) which, upon sufficient exposure and/or accumulation in the body (32,33), can exert adverse effects. Although having no physiological function, its toxic effects on humans and animals have long been known, affecting almost all organs and systems in the human body.

The effects of lead on human health depend on plasma levels and on the duration of exposure. Blood lead levels up to 29 µg/dL (1 to 1.4 µM) led to a 46% increase in all-cause mortality, a 39% increase in circulatory mortality and a 68% increase in cancer mortality (34). In addition, chronic exposure to low levels of lead causes hypertension in animals and humans (33,34). Vupputuri et al. (35), when re-evaluating the results of the Third National Health and Nutrition Examination Survey (36), reported a positive correlation between plasma lead concentration and arterial pressure in black men and women.

The limit of blood lead concentration recommended by the Agency for Toxic Substances and Disease Registry (31) is 60 µg/dL in occupationally exposed adults. However, an increase in arterial pressure has been reported for individuals with blood lead concentrations of 31.4 and 53.5 µg/dL (35-37). Previous studies on lead toxicity have reported 42.5 ± 2.3 and 58.7 µg/dL (38,39), blood concentrations that are similar to those found in the occupationally exposed population. In Brazil (Ministério do Trabalho; Norma Regulamentadora No. 7), the reference values for blood lead concentrations have been established at 40 µg/dL for unexposed persons and at 60 µg/dL for exposed persons.

A close relationship between lead exposure and hypertension has been reported in experimental studies on animals and in epidemiological reports (8). Lead-induced hypertension is reported to result from the inhibition of NKA (39) and from the reduced bioavailability of NO plus an increased endothelial release of endothelin (40,41). Free radicals reducing NO bioavailability (42) and depletion of antioxidant reserves (8,43) or their up-regulation (39) have also been reported. Peripheral or central nervous mechanisms, such as the increase of sympathetic nerve activity, the reduction of baroreflex sensitivity and the reduction of parasympathetic tone (33), are also involved in lead-induced hypertension. In addition, Carmignani et al. (32,41) reported increased myocardial inotropism and increased ACE activity in rats exposed to 60 ppm lead acetate for 10 months.

Previous studies have shown that possible mechanisms involved in lead-induced hypertension are due to alterations in vascular tone (8,39). Changes in vascular reactivity have not been extensively described in the presence of low lead concentrations and in the initial stages of lead exposure. However, studies have shown that chronic exposure to low lead concentrations induces aorta vasoconstriction (44).

On the other hand, chronic exposure to lead (100 ppm for 10 months) decreased the contractile response induced by 5-hydroxytryptamine (5-HT) in the aorta of lead-treated rats (45). These effects could be mediated by increased production of ROS and vasoconstrictor prostanoids of the cyclooxygenase pathway (26,39).

Gadolinium

Gadolinium is a trivalent lanthanide cation currently used as a magnetic resonance contrast medium, the gadobenate dimeglumine (Gd-bopta) (3), which blocks calcium channel stretch at high concentrations (10 µM) (46). However, concerns about contrast-induced nephropathy have been reported (47).

In addition to acting as a calcium channel blocker, gadolinium, at low concentration, can interact with the signaling involved in intracellular and extracellular ATP hydrolysis (48). Escalada et al. (49) reported that 3 µM gadolinium, a concentration that does not block calcium channels, has a potent inhibitory action on ecto-nucleoside triphosphate diphosphohydrolase (E-NTPDase) activity from the electric organ of *Torpedo marmorata*. Extracellular nucleotides are important molecules involved in the regulation of different biological processes including vascular tone. When released as a neurotransmitter from the sympathetic terminals, ATP binds to P2X receptors of vascular smooth muscle cells, producing vasoconstriction. When binding to endothelial P2Y receptors, ATP leads to vasodilatation (48,50). ATP exerts other effects on the vascular beds, such as control of smooth muscle and endothelial cell proliferation (48,50). The action of extracellular nucleotides is terminated by the E-NTPDase family. NTPDase1 is the major ectonucleotidase expressed in the vasculature (48,50) and its action limits platelet activation by ATP hydrolysis (48,50). NTPDase2 is another ectonucleotidase associated with the vasculature that preferentially converts ATP to ADP (50). Following the action of E-NTPDases, ecto-5'-nucleotidase is responsible for the end of nucleotide signaling by converting AMP to adenosine (51).

Gadolinium also affects ACE activity as a chelate by a transmetallation effect with zinc (52). This enzyme is a metallopeptidase containing zinc and therefore the possibility of a transmetallation effect is likely. Angiotensin II resulting from the action of ACE is the major effector of the renin-angiotensin system (RAS) (53). It activates the AT₁ receptor, has vasoconstriction and pressor effects and also causes thrombosis, inflammation and vascular and myocardial hypertrophy (53).

Interestingly, despite these actions, gadolinium has not been defined as a toxic agent. There is no toxicological information and recommended exposure limits have not been defined.

After describing the main characteristics of mercury, lead and gadolinium and considering that mercury and lead

are known to produce hypertension and gadolinium affects the RAS, we will focus on actions of these three metals on vascular reactivity. Their effects will be briefly described in protocols that are commonly employed in several laboratories mainly using vascular rings.

Effects of heavy metals on vascular reactivity

Changes in vascular responses to phenylephrine

We used the following protocol to test the general characteristics of vascular reactivity. Rats are anesthetized with sodium thiopental (50 mg/kg, *ip*) and killed by exsanguination. Thoracic aortas are removed and placed in cold oxygenated Krebs-Hanseleit bicarbonate buffer (KHB), carefully dissected out and cleaned of fat and connective tissue. For reactivity experiments, the aortas are divided into cylindrical segments 4 mm in length and cut into rings 4-6 mm in length. Rings are then mounted between parallel wires in tissue baths at 37°C containing KHB gassed with 95% O₂ and 5% CO₂ to maintain the pH at 7.4. However, for lead (as lead acetate) the bicarbonate buffer cannot be used because it precipitates. pH can be adjusted with 20 mM HEPES plus 4 mM NaOH. Rings are stretched to an optimal resting tension of 1 g and isometric tension is recorded using an isometric force displacement transducer. After 45-min equilibration each aortic ring is exposed twice to 75 mM KCl to obtain its maximum contractility. Rings are then sequentially washed and re-equilibrated and allowed to relax to baseline. Thirty minutes later, the rings are contracted with 1 μM phenylephrine and 10 μM acetylcholine (ACh) is then added to assess the integrity of the endothelium. A relaxation equal to or greater than 90% indicates functional integrity of the endothelium. Smooth muscle viability is tested using sodium nitroprusside (SNP, 0.1 mg/mL) in rings previously contracted with phenylephrine. After 30 min, cumulative concentration-response curves for phenylephrine (0.1 nM-300 μM) are generated.

The incubation of segments with mercury (HgCl₂) for 60 min promotes an increase in the reactivity to phenylephrine both at high (0.5 to 10 μM) and low (6 nM) concentrations (54,55). This increased reactivity appears as an increased R_{max} and sensitivity. Gadolinium (3 μM as GdCl₃) promotes similar responses (56) but lead increases vascular reactivity when administered only at high concentration (100 μM). At lower lead concentration (5 nM), reactivity to phenylephrine decreases.

When controlled chronic mercury treatments are performed using exposure to low doses for 30 days, with a blood concentration of 8 ng/mL (~29 nM) being reached, vascular reactivity to phenylephrine increases in Hg-treated rats (25,26). Mercury treatment also increases 5-HT-induced vasoconstriction in coronary arteries (27). A similar increase of vascular reactivity was obtained with lead treatments for 30 days or more (57). However, exposure of rats to low

lead concentrations for 7 days decreased the contractile response of aortic rings to phenylephrine (58). These findings confirm previous reports clearly suggesting that the effects of lead are concentration and time dependent (32). Regarding gadolinium, there are no reports about chronic treatments with low concentrations.

Role of the endothelium in the effects of mercury, lead and gadolinium

To evaluate its role in the vascular effects of heavy metals, the endothelium is removed by gently rubbing the intimal surface with a stainless steel rod. The effectiveness of endothelial removal is confirmed by the absence of the relaxation induced by 10 μM Ach in aortic segments precontracted with 1 μM phenylephrine. Removal is considered to be adequate when Ach-induced relaxation is less than 10% or a contraction is observed.

Removal of the endothelium increases vasoconstrictor responses of rat aortic segments to phenylephrine. This indicates that the vascular response to phenylephrine is negatively modulated by the endothelium. The effects on the phenylephrine response of all three metals, acutely administered, were dependent on the integrity of this vascular layer. Thus, when the endothelium is removed their actions are abolished (25,56,57). This endothelial dependence is observed for low and high concentrations of mercury and lead. The only effect reported thus far for gadolinium was obtained with a concentration of 3 μM (56).

The effects on the phenylephrine response observed with chronic administration of low concentrations of mercury and lead also show endothelial dependence because endothelial removal abolishes the effects of both metals. The effects of mercury and gadolinium, which increase vascular reactivity to phenylephrine, are abolished by endothelium removal and the reduced vascular reactivity to phenylephrine observed for lead is also abolished.

Effects of mercury, lead and gadolinium on endothelial modulation of vascular responses to NO

The NOS inhibitor NG-nitro-L-arginine methyl ester (L-NAME, 100 μM) is commonly used to test the endothelial modulation of vascular responses to NO. L-NAME has a potentiating effect on the vasoconstrictor response to phenylephrine, which indicates the negative endothelial NO modulation of such response. The results also give information about NO bioavailability. This is important to consider because we can have an enhanced NO production which, however, can be destroyed, reducing bioavailability. To better explain this issue some protocols can be performed such as analysis of production blockade, NO production stimulated with Ach, definition of which NOS (eNOS, nNOS, and iNOS) are involved, and which factors are destroying NO.

Acute administration of 6 nM HgCl₂ reduces the negative endothelial modulation of phenylephrine responses

(54). However, the endothelial modulation is increased after acute lead treatment, suggesting that the endothelium tries to adapt to the new environmental conditions and that the increased reactivity depends on factors other than NO production. Regarding the effects of gadolinium, L-NAME treatment in the presence of gadolinium does not change the reactivity to phenylephrine. This finding suggests that the increased vascular reactivity increment does not depend on NO but depends on another endothelial-derived factor.

Chronic treatments have been reported for mercury and lead but not for gadolinium. Mercury reduces the effect of L-NAME effect, as seen with endothelial denudation. However, lead treatment for 7 days enhanced endothelial modulation by NO, which would explain the reduced phenylephrine responses observed after lead treatment. Our findings suggest that this increased NO production is dependent on an increased activity of eNOS and iNOS.

Effects of mercury, lead and gadolinium on the endothelial modulation of vascular responses by prostanoids

Prostaglandins are endothelial factors that participate in vascular responses. The involvement of prostanoids in phenylephrine responses was investigated using 10 μM indomethacin, a well-known non-selective COX inhibitor.

Indomethacin reverses the increased reactivity to phenylephrine induced by acute administration of 6 nM HgCl_2 (54). Chronic treatment also induces the same response caused by an increased production of contractile prostanoids (prostaglandin 2 and thromboxane A2) derived from COX-2.

Similar effects are also promoted by a high concentration of lead acetate (100 μM). However, under low lead concentrations indomethacin does not alter the pressor effects of phenylephrine, suggesting that prostanoids do not play a role in the actions of lead (58). The effects of gadolinium on COX activity have not been described.

Effects of mercury, lead and gadolinium on endothelial modulation of vascular responses to endothelial-derived hyperpolarizing factors

Endothelial-derived hyperpolarizing factors (EDHFs) are important vasodilator agents that act by increasing the permeability of K^+ channels. Tetraethylammonium (TEA, 1 to 5 mM) is commonly used to test the dependence on hyperpolarizing factors. TEA blocks several potassium channels and is used as a first attempt to define the role of these channels in an intervention with toxic metals, for example. Instead of a single one, several compounds are considered to be EDHFs. NO, epoxyeicosatrienoic acids, anandamide, and even H_2O_2 have been described as EDHFs depending on the vascular bed studied.

Mercury and lead indirectly affect EDHF production because both can reduce NO bioavailability, inducing free radical generation (8,25,26). A short-term exposure to low

lead concentrations, however, reduces vascular reactivity and seems to stimulate K^+ channels in vascular smooth muscle cells (58). Again, the effects of gadolinium on EDHF activity have not been described.

Role of free radicals in the effects of mercury, lead and gadolinium on vascular responses

Several compounds can be used to investigate the possibility that free radicals play a role in the effects of mercury, lead or gadolinium on the pressure response to phenylephrine: tempol, an agent that mimics superoxide dismutase (4-hydroxy-tempo, 1 μM); deferoxamine, a free radicals scavenger (300 μM); the superoxide anion scavenger superoxide dismutase (SOD, 150 U/mL); catalase (1000 U/mL), a scavenger of H_2O_2 , and the NAD(P)H oxidase inhibitor apocynin (0.3 mM). The oxidative fluorescent dye dihydroethidium (DHE) is used to evaluate $\text{O}_2^{\cdot-}$ production *in situ* (59). Inside the cells, hydroethidine is oxidized to ethidium bromide in the presence of $\text{O}_2^{\cdot-}$ because it freely permeates cells and is then trapped by intercalation with DNA.

Mercury at low and high concentrations stimulates free radical generation after both acute and chronic treatment. This increased free radical generation is usually responsible for the reduction of NO bioavailability. Thus, we have observed that: 1) vascular $\text{O}_2^{\cdot-}$ production and plasma oxidative stress are greater in rats treated with mercury chloride, 2) mercury treatment also increases vascular mRNA levels of the NAD(P)H oxidase subunits NOX-1 and NOX-4 (27), and 3) several antioxidants abolish the increased vasoconstrictor responses observed in vessels from mercury-treated rats (25,27).

Lead is also described as an inducer of free radical production (8). The metal increases superoxide production and the blockade of its effects by SOD, catalase and apocynin shows a reduction of reactivity in the dose-response curve to phenylephrine. These findings also suggest that NADPH oxidase is also a putative site for the effect of lead in increasing free radical generation.

Effects of mercury, lead and gadolinium on ACE activity

The RAS plays an important role in the regulation of the cardiovascular system. The RAS was initially described as systemic RAS but a local RAS has been recently described in several tissues. The classic RAS depends on the action of renin. This enzyme changes angiotensinogen to angiotensin I that is converted to angiotensin II by ACE. ACE is an enzyme that has two zinc atoms in its molecule, which can be changed by other metals by a transmetallation process.

The participation of the RAS in the concentration-response curve to phenylephrine was studied in control or treated preparations by the blockade of ACE or the AT_1 angiotensin II receptor with 1 μM enalaprilate and 10 μM losartan, respectively. When the AT_1 receptor is blocked,

angiotensin II is present and its effects on AT₂ receptors might take place because they are operational. When ACE is blocked by enalaprilate, no angiotensin II is produced. Then, if the action of the metals is blocked, it suggests that ACE is being stimulated by the metals. The effects of all three metals are blocked when both drugs are used.

A curious finding is that when low concentrations of these metals are used ACE is stimulated. However, when high concentrations of mercury and gadolinium are used ACE is blocked (52). These effects have one interesting explanation, i.e., the transmetallation phenomenon (52). ACE has zinc attached to both active sites, which can be changed by other metals. The fact that at higher concentrations ACE is inhibited is also explained by another phenomenon called hormesis (60). Hormesis explains why toxic agents and venoms can activate or reduce their effects and as concentrations change the effects turn in the opposite direction.

As a final remark, the findings presented here suggest

that mercury, lead and gadolinium, even at low doses or concentrations, affect vascular reactivity. Their actions are mediated by the endothelium and result mainly from an enhanced production of free radicals and an increased production of angiotensin II by stimulation of the local ACE. The findings reported here provide further evidence that toxic metals, even at low doses, could be an environmental risk factor for cardiovascular disease.

These results also suggest that continuous exposure to these metals followed by their absorption may be hazardous to cardiovascular function and the current reference values, considered to be without risk, need to be reduced.

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