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Molecular Basis for Mercury-Induced Alteration in Endothelial Function: NO and its Modulators

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Abstract

Epidemiological and animal studies have suggested a strong association between the environmental and occupational exposure to mercury and risk of cardiovascular diseases (CVD). One of the triggering factors of CVD is endothelial dysfunction. The endothelium can evoke relaxations and contractions of the underlying smooth muscle, by releasing vasoactive agents. Nitric oxide (NO), formed by endothelial NO synthase (eNOS), is the best characterized endothelium derived relaxing factor (EDRF). The release of NO is down regulated/upregulated by factors like oxidative stress, estrogen and diseases like diabetes and hypercholelestrolemia, etc. The inhibition/activation of eNOS by mercury, affecting the NO release is one of the proposed mechanisms for mercury-induced vascular diseases. In addition, during exposure to mercury, overproduction of reactive oxygen species (ROS) can occur, resulting in oxidative stress, which is another major risk factor for endothelial dysfunction. In this article, molecular basis for mercury-mediated alteration in endothelium derived vasodilator (NO) and factors modulating the release of NO are being reviewed.

Background

For many years, the vascular endothelium, a monolayer of cells covering the vascular lumen was thought to be relatively inert. It is now recognized, as metabolically active with important paracrine, endocrine and autocrine functions, requisite for the maintenance of vascular homeostasis under physiological conditions [1,2]. The endothelium plays a vital role in the regulation of vascular tone, controlling tissue blood flow and inflammatory responses and maintaining blood fluidity [3-5]. Endothelium releases various vasoactive substances: vasodilators (NO, prostacyclin, endothelium–derived hyperpolarizing factor (EDHF), bradykinin, adrenomedullin, C–natriuretic peptide) and vasoconstrictors (endothelin–1, angiotensin–II, thromboxane A2, prostaglandins, hydrogen peroxide (H_2O_2) and free radicals) which help in regulating the vascular tone [6]. As a major regulator of vascular homeostasis, the endothelium maintains the balance between vasodilation and vasoconstriction and disturbing this tightly regulated equilibrium leads to endothelial dysfunction [7].

There is a growing data (epidemiological and animal studies) associating mercury exposure with higher risk of CVD which has been thoroughly documented [8-18]. One of the events that triggers development of cardiovascular disease is functional disruption of the vascular endothelium. Man has been exposed to mercury for centuries as it occurs naturally in the environment, and anthropogenic activities causes the release of this element into the environment, leading to pollution of air, water, and soil [19,20]. Its exposure is the second–most common cause of heavy metal poisoning. Toxicity from mercury is associated with *in-vivo* oxidative stress. The loss of endothelial function due to oxidative stress is one of the most commonly observed vascular effects of mercury exposure [21-24].

This review investigates and appraises the impact of mercury exposure on endothelium derived vasodilator, NO, highlighting the molecular basis for mercury-mediated alteration on endothelial function and modulators of NO.

Vascular Function, NO and Mercury

Nitric oxide

The L–arginine–NO pathway is thought to be the most important enzymatic vasodilator source. In addition to its function as a vasodilator, NO released from endothelial cells is also a potential inhibitor of the aggregation and adhesion of platelets to the vascular wall.

The endothelial cells synthesize NO using substrate L–arginine with help of an enzyme eNOS (endothelial nitric oxide synthase). The by-product of the reaction, l-citrulline, inhibits arginase II, which cause hydrolysis of arginine [7]. eNOS is an NADPH–dependent oxygenase that requires tetrahydrobiopterin, flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN) as cofactors [25,26]. In endothelial cells, the enzyme is localized preferentially in caveolae following post–translational acylation [27,28]. NO is formed when eNOS is stimulated by agonists such as bradykinin, acetylcholine and shear stress. Stimulation of endothelial cells causes the dissociation of the caveolin/NOS complex. This dissociation of caveolin/NOS causes binding and activation of Ca^{2+}/calmodulin and NO synthesis by eNOS [27,28]. During, shear stress activation of NOS is Ca^{2+}–independent and protein tyrosine kinase–dependent [29]. NO relaxes the vascular smooth muscle by stimulating of soluble guanylate cyclase which results in an increased formation of cyclic GMP (cGMP) [30]. The cGMP activates cGMP –dependent protein kinase which results in an increased extrusion of Ca^{2+} from the cytosol in vascular smooth

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muscle, and the inhibition of the contractile machinery [31]. Cyclic GMP–dependent protein kinase also phosphorylates K^+_{ATP} channels which culminates into hyperpolarization and thereby inhibition of vasoconstriction [32]. In certain arteries, NO directly activates K^+_{ATP} channels independently of cGMP [33] (Figure 1).

Mercury exposure and NO signaling

NO release from vascular endothelium is affected by mercury exposure (Figure 2) was reported for the first time by Golpon and co–workers [34]. They observed that in the isolated aortic tissue, low concentrations of mercury produced an endothelium dependent vasorelaxation which was blocked by the NOS inhibitor L–NAME. However at higher concentrations, mercury altered the structure and function of vascular endothelium and vasoconstriction was observed [34]. Further studies from our group on isolated aortic rings validated that mercury produces a dual response: vasoconstriction at high concentration and vasorelaxation at lower concentrations. L–NAME and glybenclamide block the vasorelaxation produced at low concentrations of mercury suggesting that mercury acts through activation of NOS and K^+_{ATP} channel [35]. It may be hypothesized that mercury stimulates NOS, which forms NO and consequently the synthesis of cGMP. cGMP increases extrusion of Ca^{2+} from the cytosol in vascular smooth muscle, causing vasorelaxation [35]. The cGMP and NO also directly phosphorylate K^+_{ATP} channels. K^+_{ATP} channels induce hyperpolarization, which results also in vasorelaxation [31,32]. Decreased acetylcholine (ACh) induced vasorelaxation has been observed when rat aortic rings were exposed to mercury chloride (10^{-5} M) and methyl mercury chloride (10^{-5} M) separately *in-vitro* suggesting endothelial dysfunction. However, an increased ACh induced vasodilation was observed in rat aortic rings exposed to low concentrations of mercury chloride (10^{-9} M), and methyl mercury chloride (10^{-9} M), separately indicating modulation of the endothelial function. The NOS inhibitor, L-NAME significantly reduced the ACh induced vasorelaxation in mercury chloride (10^{-9} M) and methyl mercury chloride (10^{-9} M) exposed aortic rings, in comparison to mercury chloride (10^{-5} M) and methyl mercury chloride (10^{-5} M) exposed aortic rings. Mercury induces increased/decreased production of NO depending on the dose of exposure [36]. The above experiments by Golpon and our group were performed on isolated vascular tissue where other physiological mechanisms do not play any role. In an animal model many factors get involved, the effects of mercury on vascular function especially those on NO and factors modulating the release of NO are interesting.

Mercury and modulation of protective role of Nitric oxide

The ability of the endothelium to release NO can be upregulated/downregulated by several factors. NO is upregulated by shear stress, estrogen, insulin, adiponectin, aldosterone, arginine and ω unsaturated fatty acid, etc. NO is downregulated by oxidative stress, aging, obesity, hypercholesterolemisa etc [7]. Several studies have shown a connection between mercury exposure and factors which may upregulate and down regulate the release of endothelium from NO.

Reactive oxygen species

Several enzymes in the endothelial cells namely NADPH oxidase, xanthine oxidase, cyclooxygenase and eNOS itself generate super-oxide anions [7]. eNOS, generates super-oxide anions when it is uncoupled by lack of substrate (l-arginine) or shortage of the essential co-factor tetrahydrobiopterin (BH4) [37]. Super-oxide anions are dismutated to hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD). H_2O_2 contribute to endothelium-dependent relaxations or is broken down by catalase [3,38]. However, superoxide anions also react avidly with NO resulting in the formation of a potent vasoconstrictor peroxynitrite [37,39,40]. Superoxide anion also reduces the bioavailability of NO resulting in reduced endothelium-dependent relaxations [39] (Figure 1). Antioxidants have shown to acutely improve endothelial responses *in-vitro* and *in-vivo* both in animals and humans [41-44].

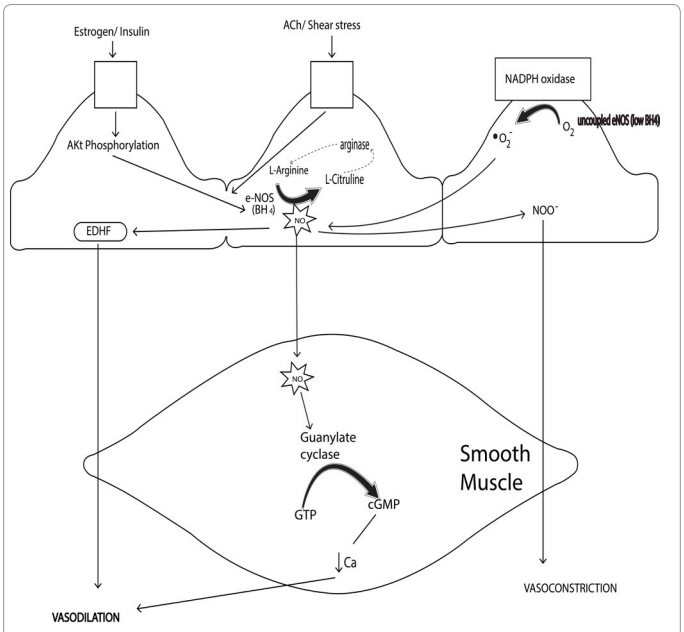

Figure 1: Schematic representation of possible mechanisms (only those mechanism affected by mercury exposure) by which production of nitric oxide is regulated in endothelial cells. Nitric oxide is produced through enzymatic conversion of l-arginine by nitric oxide synthase (eNOS). Insulin and estrogen cause upregulation of eNOS by Akt phosphorylation. Oxidative stress causes downregulation of eNOS due to low tetrahydrobiopterin (BH_4). The by-product of the eNOS reaction, l-citrulline (l-Cit), inhibits arginase which hydrolyses arginine.

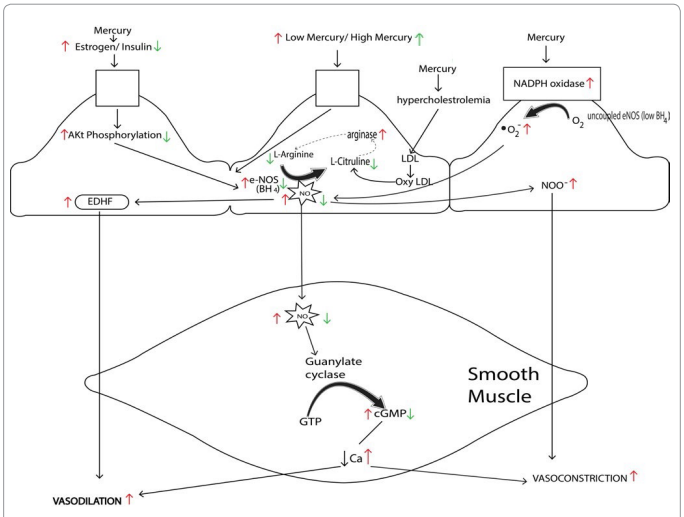

Figure 2: Schematic representation of effect of low/high mercury exposure on endothelium-derived vasoactive factors. In response to low mercury exposure, an increase in the eNOS activity/expression causes an increase in NO. In response to high mercury exposure decrease in the eNOS activity/expression causes decrease in NO with persistence or upregulation of EDHF. Mercury exposure also causes oxidative stress, hypercholesterolemia, decrease in insulin and affects estrogen.

There are a number of studies revealing that mercury exposure generates oxygen radicals, with subsequent oxidative damage in several organs and systems as well as alters the antioxidant defense system in the cells [45-49]. Cell toxicity caused by heavy metal ions is attributed to oxidative and nitrosative stress, defined as an excess of oxidants over antioxidants. Macromolecules in cells are damaged by mercury–induced production of oxygen and nitrogen–containing free radicals (oxidants) and/or metal–induced depletion of the antioxidant defences. The vascular endothelium is very sensitive to oxidative stress [50-52]. Although multiple processes may lead to endothelial damage, the generation of oxygen–derived free radicals and subsequent lipid peroxidation may be one of the key components in the cascade of events.

On exposure to mercury, vascular Endothelial cells (ECs) in culture, generates superoxide anions (oxidative stress) in ECs. Mercury induced oxidative stress in ECs is caused by the activation of phospholipase D (PLD) which results in generation of, 2-diacylglycerol (DAG), a second messenger for vasoconstriction [53]. Similarly in Bovine Pulmonary Artery Endothelial Cells (BPAECs), mercury-induced PLD activity is attenuated by L-type calcium channel blockers demonstrating the importance of calcium and calmodulin in the regulation of mercury-induced PLD activation and the protective action of L-type calcium channel blockers against mercury cytotoxicity in vascular ECs [54]. In bovine pulmonary artery endothelial cell monolayers, it has been reported that mercury ions induce oxidative stress through depletion of GSH and inactivation of thiol enzymes [55].

Studies on isolated aortic rings indicate that the dual response (vasoconstriction at high concentration and vasorelaxation at lower concentrations) produced by mercury is ameliorated by antioxidants SOD and catalase and L-type Ca channel [35].

In another *in-vitro* mercury exposure ($HgCl_2$, 6 nM) study, mercury induced modulation of vascular reactivity was observed due to increased release of ROS derived from NADPH oxidase which culminated into reduced bioavailability of NO [56]. Mercury causes contraction of the vascular smooth muscle in isolated rat vascular bed which is mediated by the formation of superoxide anions and by reduction in the endothelial vasodilator activity [57].

Acute and chronic exposure to mercury in rats causes an increase in vascular O^{2-} production, plasma malondialdehyde levels and total antioxidant [35,55,57-61]. Low dose of mercury exposure causes endothelial dysfunction and alters the coronary vascular reactivity. This alteration is partially, due to increase in both NOX-1 and NOX-4 subunits suggesting the involvement of NADPH oxidase resulting in increased ROS production. A decrease in antioxidant defenses could also be a contributory factor to the increased superoxide production observed after mercury treatment [60]. In contrast several authors have reported augmented antioxidant defenses to protect cells against the increased oxidative stress after acute and chronic mercury exposure [55,56,62,63].

The interplay between NO and oxidative stress occurs on mercury exposure as free radical scavengers along with L-NAME blocks the mercury induced vasorelaxant or vasoconstrictor response in isolated aortic rings. SOD þ catalase blocks the ROS mediated vasoconstrictor response and L-NAME blocks the NO-mediated vasorelaxant response. Mercury evokes the production of both NO and ROS, and it is their percentage that produces the response. If ROS produced is more than NO, peroxynitrite is formed and a vasoconstriction is produced [35].

This interaction of NO and oxidative stress was further validated by an acute exposure study of methyl mercury chloride (5 mg/kg; po.) in rats. Oxidative stress was produced along with an increase in serum NO levels. A significant increase in the acetylcholine vasodilator response in isolated aortic rings from mercury exposed rats was observed. This effect was mediated by increased production of NO, because of stimulation of eNOS. It is interesting to note that this increase occurred even when there was oxidative stress, suggesting a state of inclination towards NO [63] (Figure 2).

Contrary, to above an opposite interaction between NO and oxidative stress was observed in a chronic study on healthy wistar rats exposed to inorganic mercuric chloride in drinking water for 30 days. Oxidative stress accompanied with increased NO levels and endothelial dysfunction, was observed. Even though there was an increase in the serum NO level, endothelial dysfunction was detected. As there was a significant increase in free radical production, the free radicals must have interacted with NO and reduced the bioavailability of NO resulting in endothelial dysfunction. The EDHF pathway was relatively resistant to mercury exposure and oxidative stress, suggesting that there may be an up-regulation of K^+_{ATP} channels in order to maintain circulation to compensate the attenuated NO-mediated vasodilatation (Figure 2) [58].

The evidence available suggests that antioxidants may play an important role in abating some health hazards (endothelial dysfunction) of mercury exposure.

Insulin

In-vitro studies on insulin have recorded an enhancement in the expression of eNOS in native endothelial cells *in- vitro* [64] and facilitation of NO-dependent vasorelaxations *in-vivo* [65-67] suggesting that NO is upregulated in presence of insulin (Figure 1). Decrease in insulin may result in attenuation in endothelium release of NO. There is a significant correlation between diabetes (impaired/decreased insulin secretion) and total hair mercury levels [68-71]. Follow up studies of patients with Minamata disease (organic mercury poisoning) in Japan, have also shown an increased incidence of diabetes 1 due to pancreatic β-cells dysfunction [72]. Similarly, *in-vitro* studies have furthermore shown a mercury dependent decrease in the function and viability of pancreatic β-cells due to increased oxidative stress, causing impaired insulin secretion [73]. One of the reasons of endothelial dysfunction in mercury exposure worth characterizing may be decrease in insulin levels leading to downregulation of eNOS and endothelial dysfunction (Figure 2).

Estrogen

The potentiating effect of estrogens on endothelium-dependent relaxations involves both genomic and non-genomic effects [74-76]. It depends presumably both on a reduction in oxidative stress leading to an increased bioavailability of NO and an increased responsiveness of the vascular smooth muscle cells to vasodilator stimuli [76-79] (Figure 1). Rowland et al. (1994) reported that 418 women with high exposure to mercury (i.e., female dental assistants) were less fertile than unexposed controls [80]. In a study by Baranski and Szymczyk (1973), female rats exposed via inhalation to metallic mercury (at an average of 2.5 mg/m^3, 6 hours a day, 5 days a week for 21 days) experienced longer estrous cycles than unexposed animals [81]. Decrease in estrogen level after mercury exposure renders testis more susceptible to oxidative damage leading to its functional inactivation [82]. Mercuric chloride also exhibits estrogen-like effect through binding and activating estrogen receptor (ER) [83]. It is likely that the potentiating effect of mercury on NO release presumably may be due to its estrogen like

effect and further studies are needed to test the hypothesis (Figure 2).

Omega-3 Unsaturated Fatty Acid

Consumption of fish has always been considered beneficial for the health of the cardiovascular system. The benefits of fish are mainly due to its content of omega-3 long chain polyunsaturated fatty acids (n-3 PUFA), including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Fish consumption is associated with decreased circulating biomarkers of endothelial dysfunction and inflammation [84], and flow mediated dilation values seem to improve after n-3 PUFA intake [85] The chronic intake of Omega-3-unsaturated fatty acids potentiates the endothelium-dependent relaxations of coronary arteries [86-88]. Omega-3 fatty acid might attenuate Mercury chloride-induced toxicity by improving antioxidant status and acute phase response in mice [89]. There are a contrary number of studies, that say the consumption of fish contaminated with mercury, causes high mercury levels in blood, hair, urine and toe nail, which diminishes/does not diminish its cardio protective effect [90-94]. Omega 3-fatty acids attenuates vascular endothelial dysfunction, by activating eNOS through opening of K^+_{ATP} channels in sodium arsenite exposed rats [95]. As mercury is known to modulate eNOS and also K^+_{ATP} channels the beneficial effect of habitual consumption of high amounts of fish is associated with better endothelial function despite higher serum concentrations of mercury [96].

Hypercholesterolemia

Hypercholesterolemia reduces endothelium-dependent relaxations due to increased oxidative stress leading to a reduced bioavailability of NO [97-100]. Long-term MeHg treatment induces dyslipidemia, characterized by increased serum cholesterol levels in mice [101]. In a study done on 274 school children, an increased level of mercury in urine was associated with elevated cholesterol level which is a known risk factor of myocardial infarction, coronary disease, and cardiovascular disease [102]. Hypercholesterolemia reduces eNOS activity and may act as a triggering factor of mercury induced reduction in endothelium dependent relaxation (Figure 2).

Conclusion

Vascular endothelial cells respond to low concentration of mercury by releasing NO, which relaxes the vascular smooth muscle that surrounds them. High concentration of mercury causes decrease in endothelial NO and vasoconstriction. Decrease/increase in release of NO on mercury exposure is majorly regulated by superoxide anions and minorly may be by insulin, estrogen, omega-3 unsaturated fatty acid and hypercholesterolemia. It emerges that a fragile equilibrium exists between NO released and superoxide anions generated by mercury exposure. When this balance leans towards NO, enhanced endothelial function is detected. When the equilibrium inclines in favour of oxidative stress, endothelial dysfunction is observed. The endothelial dysfunction caused by the imbalance between the production of NO and oxidative stress on mercury exposure can be compensated by EDHF-mediated vasorelaxation. Mercury exposure affects insulin (decrease/impaired) and causes hypercholesterolemia, downregulating the release of NO. Mercury has estrogen like affect and may be increasing the release of NO. The beneficial effects of omega-3 unsaturated fatty acid on mercury exposure and endothelial vascular release of NO are a point of contention. NO signalling mechanism and oxidative stress play a vital function in the mercury-induced cardiovascular diseases in the populations exposed to mercury.

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