PERSPECTIVE

Mercury-induced neurotoxicity and neuroprotective effects of berberine

Exposure to mercury can cause immune, sensory, neurological, motor and behavioral dysfunctions similar to traits associated with autism spectrum disorders (ASDs), and these similarities extend to neuroanatomy, neurotransmitters and biochemistry. It also affects antioxidant system in the cell, resulting in loss of membrane integrity and finally cellular necrosis (Abdel Moneim, 2015).

Mercury (Hg) is a heavy metal of known toxicity, it naturally occurs in several physical and chemical forms: elemental or metallic mercury (Hg^0), inorganic mercury [(mercurous (Hg^+) and mercuric (Hg^{2+})] and organic mercury (in which mercury is bonded to methyl, ethyl, phenyl, or similar groups), and all of it can produce toxic effects even in low doses (Abdel Moneim, 2015). Organic mercury compounds, such as methylmercury (MeHg), have been extensively studied because they are able to reach high levels in the central nervous system (CNS), leading to neurotoxicity. On entry to the bloodstream, MeHg adheres to sulfhydryl groups, particularly to those in cysteine, and it deposited throughout the body (Bernhoft, 2012).

Deposited MeHg slowly undergoes demethylation to inorganic mercury that cannot normally penetrate the blood brain barrier by the L-type neutral amino acid carrier transport (LAT) system. After then, Hg is trapped inside the brain without the ability to way back. The mechanism by which Hg induced neurological damage is still unclear. However, Hg is able to disrupt cell cycle progression and/or induce apoptosis in several tissues is well recognized. Moreover, Hg-induced neurotoxicity is known to be mediated by reactive oxygen species (ROS) in different models by alternating Na^+/K^+ ATPase activity and mitochondrial function (Mieiro et al., 2011). Hg leads to the depletion of glutathione (GSH) content as a result of Hg adheres to sulfhydryl groups, Hg interacts with the thiol group of GSH leading to the formation of an excretable GS-$HgCH_3$ complex, and all these inhibit glutathione reductase and glutathione peroxidase activities. This is frequently suggested to be an expression of Hg neurotoxicity (Mieiro et al., 2011).

Moreover, a large body of evidence has also drawn the link between Hg and glutamate-mediated excitotoxicity. For example, Hg has been shown to affect several aspects of glutamatergic signaling, including the inhibition of glutamate reuptake in astrocyte, inhibition of glutamine synthetase activity, and an enhancement of spontaneous glutamate release from neurons. As a result of these effects, glutamate concentration at the synaptic cleft is increased. MeHg has also been found to impact postsynaptic N-methyl-D-aspartate (NMDA) receptors which may increase NMDA receptor expression and/or enhance sensitivity of NMDA receptors (Xu et al., 2012). The overactivation of NMDA-type glutamate receptors increases Ca^{2+} influx into neurons, therefore leading to the activation of important pathways involved in cell death. Furthermore, Ca^{2+} stimulates ROS generation by mitochondria] (Farina et al., 2011a).

Mitochondria are one of the target organelle for Hg-induced cell death. Hg decreased mitochondrial transmembrane potential and increased reactive oxygen species (ROS) generation. The changes in mitochondrial membrane potential open the mitochondrial permeability transition pore, which leads to the release of apoptogenic factors, such as cytochrome c, component of the apoptosome that activates caspase cascade. Furthermore, Hg exposure induces a decrease in the activity of enzymes of the mitochondrial energy metabolism such as cytochrome c oxidase, superoxide dismutase and succinate dehydrogenase (do Nascimento et al., 2008).

Several lines of evidence indicate that Hg neurotoxicity is associated with increased levels of reactive (oxygen/nitrogen) species (ROS/RNS). The mechanisms mediating Hg-induced ROS/RNS generation appear to be far more complex. Hg induces a "selenium-deficient-like" condition, which affects glutathione peroxidase (GPx) synthesis and leads to decreased GPx levels, consequently, increased H_2O_2 levels. Another mechanism related to the increased H_2O_2 levels after Hg exposure appears to be the direct hampering effect of this toxicant toward the entire GSH antioxidant system thus leads to increased levels of brain H_2O_2 and lipid peroxidation (Farina et al., 2011b). Lipid peroxidation is the noxious biological event orchestrated by the free radicals such as H_2O_2 resulting in structural alterations of membranes and functional impairment of neuronal components.

Moreover, increased intracellular calcium levels are prone to activate neuronal nitric oxide synthase (nNOS), as a result of the overactivation of NMDA-type glutamate receptors, thus increasing nitric oxide (NO) formation. Another interesting and recently reported event that has been connected to the pro-oxidative effects of Hg is the activation of nuclear factor erythroid-2-related factor-2 (Nrf2) (a major regulator of intracellular antioxidant response). There is an evidence that the interaction between Nrf2 and Keap1 is disrupted by oxidative modifications

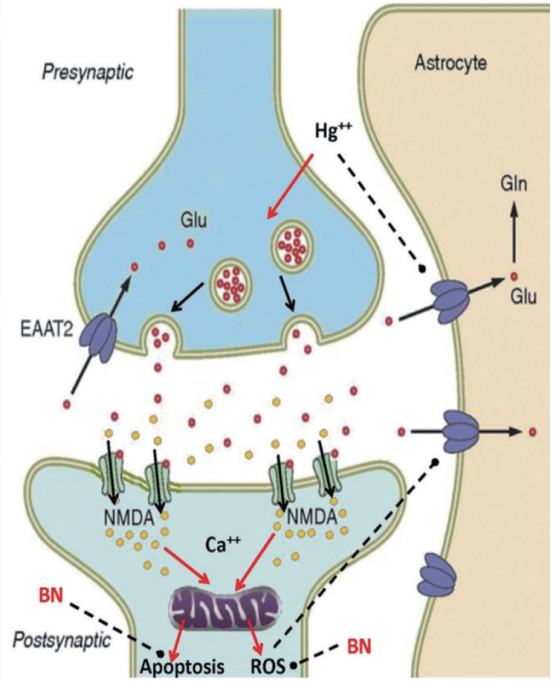

Figure 1 Mercury-induced neurotoxicity and BN attenuates mercury neurotoxicity.
Hg-induced neurotoxicity was partially elucidated by distributing glutamate and calcium homeostasis, as well as ROS generation. The three events activate neuronal death pathway. Whereas, BN possesses neuroprotection *via* antioxidant defense stimulation, increasing the anti-apoptotic signaling, and decreasing the apoptotic signaling and the NMDA receptors. BN: Berberine; Ca^{++}: calcium ion (yellow ball); EAAT2: excitatory amino-acid transporter 2; Gln: glycine; Glu: glutamate (red ball); Hg^{++}: mercury ion; NMDA: N-methyl-D-aspartate; ROS: reactive oxygen species.

of their cysteine thiol groups (Farina et al., 2011b).

Again, an increase in intracellular calcium levels triggers calcium-dependent injurious processes, an activation of degradative enzymes such as phospholipases and proteases, resulting in an irreversible damage of cellular components and eventually cell death. Phospholipases and proteases are potentially involved in cytoskeletal alterations and blebbing of the plasma membrane leading to membrane damage (Kuo and Lin-Shiau, 2004). Another mechanism related to Hg induced apoptosis is the depolarization of mitochondrial membrane. The permeabilization of the mitochondrial membrane results in the release of cytochrome c, the major steps in the mitochondrial pathway of apoptosis. The release of cytochrome c triggers the assembly of Apaf-1 (apoptotic protease-activating factor) and pro-caspase-9 to form an apoptosome. Pro-caspase-9 is then autolyticaly cleaved to active caspase-9, which then activates pro-caspase-3 to active caspase resulting in cleavage of its substrates and apoptosis. The release of cytochrome c and AIF are controlled by several members of the Bcl-2 family, especially Bax, Bcl-2, and Bcl-xL. Hg induces phosphorylation of the Bcl-2 family or alters their intracellular localization without affecting the protein levels. It is also possible that other proapoptotic molecules like Bmf (Bcl-2-modifying factor), Bik, Bak, or Bim are upregulated by Hg. AIF once released from mitochondria, is transported into the nucleus, where it stimulates (ATP-independent and caspase-independent) large DNA fragmentation and condensation of chromatin (Makani et al., 2002).

As a result of the involvement of oxidative stress and apoptosis in mercury-induced neurotoxicity, my laboratory has been investigating the neuroprotective role of berberine (BN). BN is one of protoberberine isoquinoline alkaloid extracted from the roots and barks of many plants of the Berberis species. BN has long been used as a traditional remedy as it has many medicinal properties that have attracted the attention of researchers over the last decade. These properties include anti-inflammatory, antioxidant activities (Othman et al., 2014). Due to its high blood-brain barrier permeability, the beneficial neuroprotective effect of BN in Hg exposure was studied (Wang et al., 2005).

The neuroprotective effect of BN in Hg exposure is due to a fact that BN could down-regulate the caspase-3, Bax and nuclear factor-kappa B (NF-κB) to suppress the pro-apoptosis signal. BN can also promote the phosphorylation of Bcl-2 and inhibit apoptosis *via* activation of the PI3K/Akt signaling pathway, these subsequently increase neuron survival. Moreover, BN can markedly prevent the rise in brain-lipid peroxidation by maintaining the balance of oxidant and antioxidant status and nitric oxide through the inhibition of nitric oxide synthase expression. Moreover, BN can markedly suppress oxidative stress through induction of Nrf2 pathway. Furthermore, BN has been reported to improve spatial memory impairment in Alzheimer's disease and can attenuate ischemic injury by inhibiting reactive astrogliosis and microglial activation.

In summary, Hg-induced neurotoxicity was partially elucidated. In this regard, glutamate and calcium dyshomeostasis, as well as ROS generation, are three important and interrelated phenomena that mediate a toxic cycle that culminates in neuronal death (**Figure 1**). Whereas, BN possesses neuroprotective effects *via* antioxidant defense stimulation. BN also decreases oxygen consumption and increases mitochondrial membrane potential, which improves mitochondrial function, leading to protection against oxidative stress. In addition, BN attenuates Hg-induced apoptotic neuronal cell death by increasing anti-apoptotic signaling (Bcl-2), and decreasing apoptotic signaling (Bax, cytochrome c and caspase).

Concluding remarks
- Over stimulation of NMDA receptors is likely the initial trigger for the downstream cascades participated in neurotoxicity of mercury.
- ROS/RNS generation emphasizes mercury-induced neurotoxicity.
- Mitochondria are one of the target organelle for mercury-induced cell death.
- Berberine provides neuroprotection against deleterious mercury-mediated free radical attacks.
- Berberine might decrease ROS production *via* inhibiting NMDA receptors.

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