

Review Article

Role of Calcium and Mitochondria in MeHg-Mediated Cytotoxicity

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Methylmercury (MeHg) mediated cytotoxicity is associated with loss of intracellular calcium (Ca^{2+}) homeostasis. The imbalance in Ca^{2+} physiology is believed to be associated with dysregulation of Ca^{2+} intracellular stores and/or increased permeability of the biomembranes to this ion. In this paper we summarize the contribution of glutamate dyshomeostasis in intracellular Ca^{2+} overload and highlight the mitochondrial dysfunctions induced by MeHg via Ca^{2+} overload. Mitochondrial disturbances elicited by Ca^{2+} may involve several molecular events (i.e., alterations in the activity of the mitochondrial electron transport chain complexes, mitochondrial proton gradient dissipation, mitochondrial permeability transition pore (MPTP) opening, thiol depletion, failure of energy metabolism, reactive oxygen species overproduction) that could culminate in cell death. Here we will focus on the role of oxidative stress in these phenomena. Additionally, possible antioxidant therapies that could be effective in the treatment of MeHg intoxication are briefly discussed.

1. Introduction

Mercury is one of the most studied heavy metal due to its wide distribution in nature. In the environment, humans and animals can be exposed to different chemical forms of mercury, including elemental mercury vapor (Hg^0), inorganic mercurous (Hg^{2++}), mercuric (Hg^{++}), and organic mercuric compounds as ethylmercury, methylmercury, and dimethylmercury [1, 2]. All forms of mercury can be toxic and the extent of the toxic effects varies depending on the dose, chemical form, and level of exposure. Among the organic forms, methylmercury (MeHg) is the most frequently encountered in the environment. It is formed mainly as the result of methylation of inorganic (mercuric) forms of mercury by microorganisms in aquatic milieu, where it can pass up through the aquatic food chain and bioaccumulates in fish and sea mammals [1] (Figure 1). At present, sea food consumption represents the main human exposure route for MeHg and the brain is the main target organ for its toxicity. Neurological symptoms induced by MeHg intoxication can

include cerebellar ataxia, paresthesia, dysarthria, mnemonic deficits, memory impairment, and sensory disorders [3, 4]. MeHg has become a ubiquitous pollutant since outbreak of environmental disasters that occurred in Japan (1950s) and Iraq (1970s) due the consumption of MeHg-contaminated fish and seed grain, respectively [5, 6]. Although MeHg is known to affect adult central nervous system (CNS), these catastrophic episodes revealed the particular sensitivity of immature brain to high concentration of MeHg. Epidemiological evidence also shows that acute or chronic prenatal exposure to low MeHg levels from maternal consumption of fish can cause neurological deficits in children. Cerebral palsy, mental retardation, deafness, and blindness are some of abnormalities caused by fetal and neonatal MeHg exposure [3, 4, 7, 8]. Despite these observations, there is evidence that the fetal brain is more susceptible than infantile brain to MeHg toxicity. Differences among gestation stage, exposure duration, and efficacy of antioxidant systems in developing brain might be determinant factors in the age-dependent neuronal vulnerability to MeHg [4, 8–10].

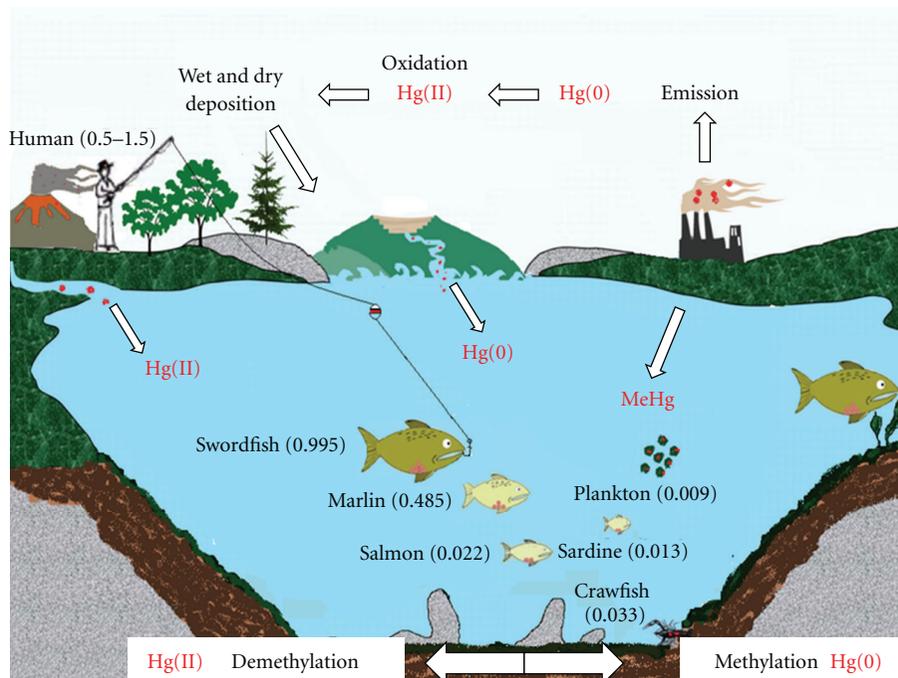


FIGURE 1: Cycle of mercury and its bioaccumulation in aquatic food chain. The values of mercury levels in the plankton and fish are represented as ppm. All data presented in this figure were obtained from (FDA and EPA).

As a consequence of high affinity of MeHg for $-SH$ groups, the molecular interaction between MeHg and sulfhydryl-containing molecules as L-cysteine, glutathione (GSH), hemoglobin, and albumin has been implicated in the mechanisms involving transport, uptake, and accumulation of MeHg into living tissues [11]. In this regard, several studies have demonstrated that the cellular uptake of MeHg is markedly increased when it is present as Cys-MeHg conjugate [12, 13] once that this complex by mimicking structurally the amino acid methionine is a substrate for the neutral amino acid carrier L-type [11, 14] (Figure 2).

Different mechanism and molecular targets have been proposed to be involved in MeHg neurotoxicity. Thiol depletion (especially glutathione), glutamate dyshomeostasis, calcium dysregulation, oxidative stress, cytoskeletal disruption, and mitochondrial dysfunctions are among the detrimental effects known to render neurons vulnerability to MeHg toxicity [15, 16] (Figure 3).

One of the most widely documented effects caused by MeHg on the CNS is associated with glutamate-mediated excitotoxicity, which can be linked to or followed by intracellular Ca^{2+} overload. In this way, there are a number of experimental findings from *in vivo* and *in vitro* studies pointing that the inhibition of glutamate uptake by astrocytes, the increase in spontaneous release of glutamate from presynaptic terminals, and the inhibition of vesicular glutamate uptake are critical phenomena linked to MeHg-mediated excitotoxicity [15, 17, 18] (Figure 4). In this scenario, the excitatory amino acid receptors (N-methyl D-aspartate (NMDA) and non-NMDA-types) mediated pathways have been indicated as the main routes responsible by Ca^{2+} entry into cells following

MeHg exposure [19, 20]. In accordance, a recent study using mouse spinal motor neurons in culture demonstrated that the excitatory amino acid receptor blockers MK-801 and AP-5 both NMDA receptor-operated ion channel blockers and the CNQX, a non-NMDA receptor blocker, were effective in delaying the development of increased Ca^{2+} after MeHg exposure [20]. In addition to glutamatergic receptors, there is evidence that voltage-dependent Ca^{2+} channels also contribute substantially to calcium influx after exposure to MeHg in neurons [20].

Regarding to a possible redox modulation of NMDA-type glutamate receptors directly by MeHg, there are no available data in the literature. However, it is important to mention here the study of Tang and Aizenman [21] showing that the alkylation of the NMDA redox site by the sulfhydryl alkylating agent N-ethylmaleimide (NEM) potentiated the response of receptor and renders NMDA receptors unresponsive to oxidation by Ellman's reagent 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB). Considering that the MeHg is a molecule as small as NEM and has high affinity by $-SH$ groups, it is plausible to suppose that part of effects induced by MeHg on glutamate dyshomeostasis may be a consequence of its interaction with the redox modulatory site of the NMDA receptor. Taken together, the events mentioned above cause sustained elevation of intracellular Ca^{2+} that may trigger cell death pathways by many different mechanisms [20]. In these circumstances is pointed the close relationship existent between Ca^{2+} overload and mitochondria dysfunctions, which plays a crucial role in regulating cellular injury induced by MeHg (Figure 4).

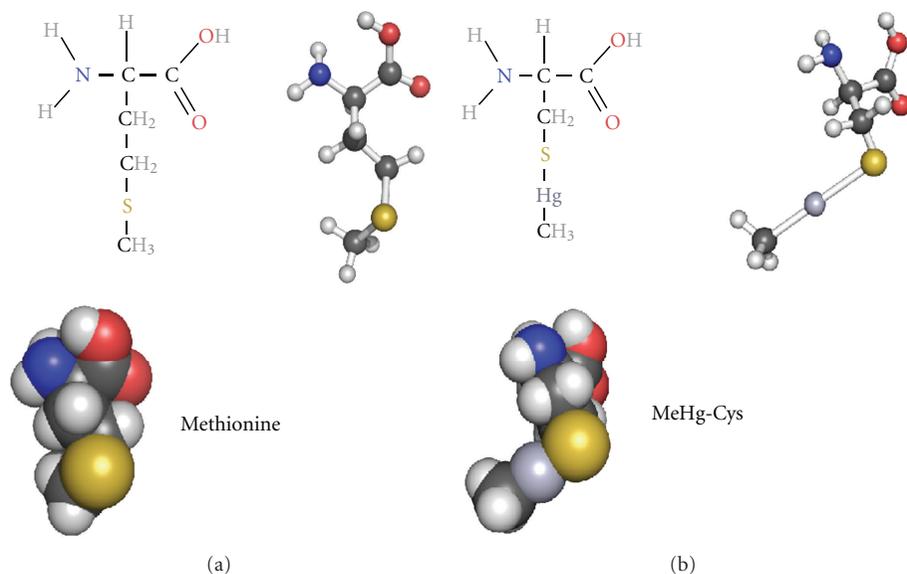


FIGURE 2: Schematic representation of the structures and space-filled models of methionine (a) and MeHg-Cys complex (b). Note the similarities in chemical structure between the MeHg-Cys conjugate and the amino acid methionine. The geometry-optimized using Universal Force Field (UFF). The representations (ball and stick, Vander Waals spheres) were obtained using the program PyMOL (Molecular Graphics System, Version 1.5.0.1, Schrödinger LLC).

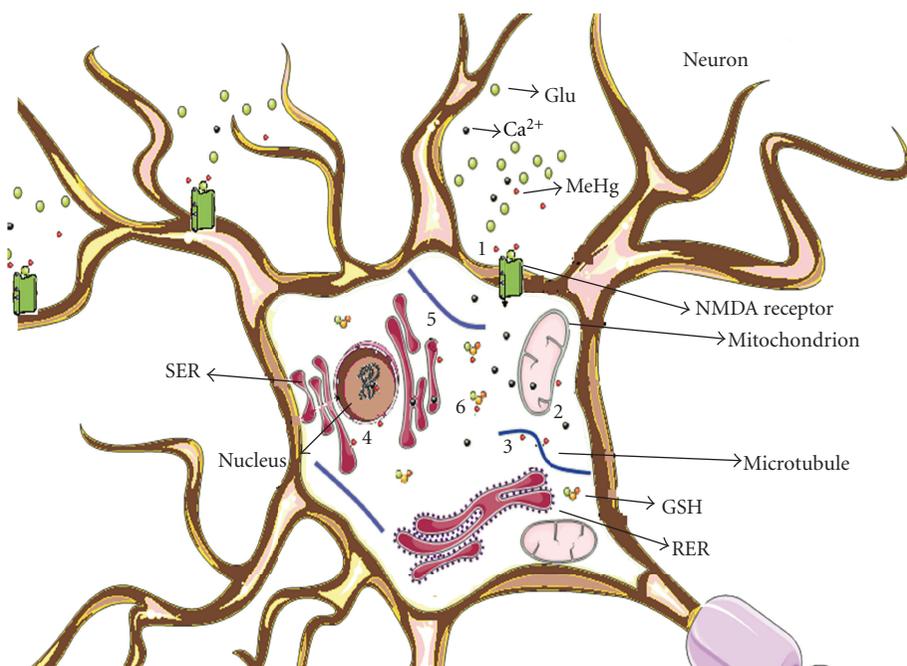


FIGURE 3: Schematic representation of possible mechanisms and cellular targets involved in the neurotoxicity MeHg-induced: (1) glutamate dysregulation and Ca²⁺ intracellular dysregulation; (2) mitochondrial dysfunction; (3) cytoskeletal disruption; (4) DNA damage; (5) SER dysfunction; (6) thiol depletion (especially glutathione). This scheme is merely representative and the scale of structures does not represent the real size.

2. Calcium Homeostasis and Mitochondria

The concentration of Ca²⁺ in the cytosol is tightly regulated in all cells because calcium is a key element in metabolic and intracellular signaling regulation. Conversely, Ca²⁺ is

also a relevant marker of numerous pathological processes when it is present at high, nonphysiological concentrations, notably neurological disorders [22]. The Ca²⁺ transport across neuronal cells membranes can occur by a variety of different mechanisms. In general, under resting conditions

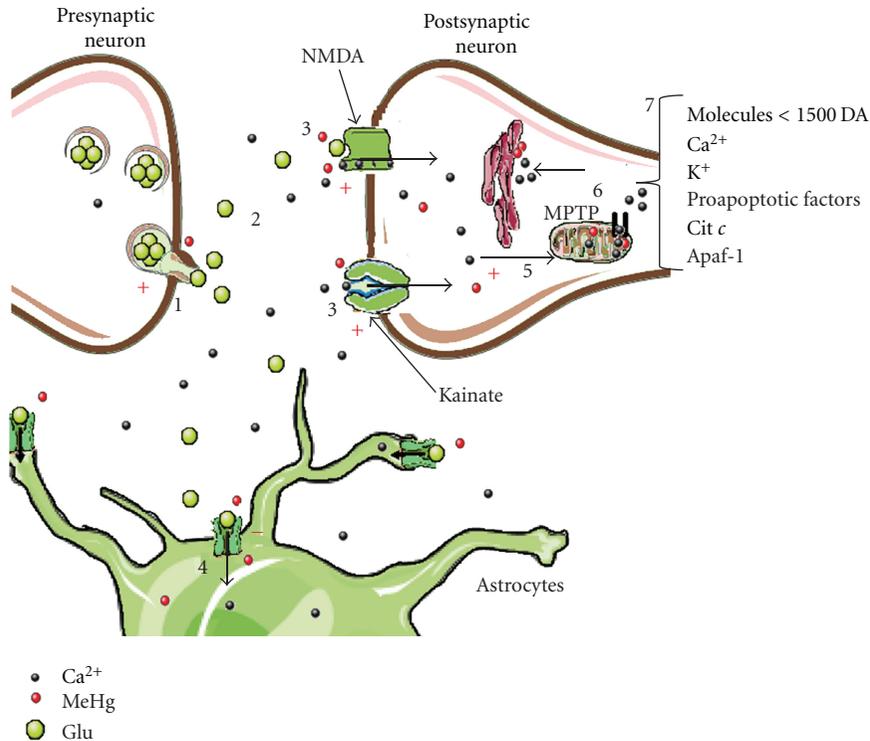


FIGURE 4: MeHg as mediator of neuronal toxicity via Ca²⁺-mediated excitotoxicity: (1) glutamate (Glu) release from presynaptic neuron induced by MeHg; (2) increase of Glu into synaptic cleft; (3) Ca²⁺ influx via NMDA and Kainate receptors; (4) MeHg binds at Glu transporters in astrocytes; (5) mitochondria buffering the excess of Ca²⁺ intracellular; (6) MeHg causing mitochondrial and SER damage; (7) MPTP opening with release of pro-apoptotic factors induced by MeHg alone and/or excess of [Ca²⁺]_i.

the intracellular Ca²⁺ levels ([Ca²⁺]_i) are maintained at very low concentrations (in the nM range) when compared to the extracellular levels ([Ca²⁺]_e) (in the mM range) [20, 22, 23]. On the other hand, in the region of transmitter release from active zones, at nerve terminals, the [Ca²⁺]_i can be many times greater, reaching, at least transiently, mM levels [20, 23–29].

During normal neuronal activity the entry of Ca²⁺ into neurons occurs very fast and without energy expenditure, since it takes place in favor of the concentration gradient across the plasma membrane. However, the restoration of [Ca²⁺]_i (following a transient increase) is much slower and depends on energy expenditure (either directly or indirectly through electrogenic “pumping” activities) [23, 25]. Therefore, the restoration of [Ca²⁺]_i homeostasis is extremely “expensive”, being the “cost” directly proportional to the cell activity (i.e., highly active cells such as neurons expend a lot of cellular energy in order to restore the Ca²⁺ homeostasis). In this condition, some organelles can act as “Ca²⁺ buffers” by sequestering the excess of Ca²⁺; however, this “storage” of Ca²⁺ also requires cost of cellular ATP equivalents [23, 30, 31] (Figure 5).

It is now well established that sustained elevations of [Ca²⁺]_i can cause neuronal degeneration and cell death by activating biochemical cascades that result in either necrotic or apoptotic processes [30–39]. However, the exact molecular mechanisms by which continuous increases in

[Ca²⁺]_i elicit neuronal cell death pathways are not fully understood. It is believed that these may include the activation of degradative enzymes, such as phospholipases, proteases and endonucleases, perturbation of cytoskeletal organization, and, primarily mitochondrial dysfunctions [22, 30, 33].

Considering the importance of maintaining appropriate intracellular concentrations of Ca²⁺ for proper cellular function and the delicate balance between the physiological and toxicological effects of [Ca²⁺]_i, in addition to mechanisms for Ca²⁺ removal from the cell, there are also critical mechanisms for the intracellular storage of Ca²⁺ in the cells. The most important cellular Ca²⁺ stores are the organelles mitochondria and smooth endoplasmic reticulum (SER). Mitochondria present low affinity and high capacity to Ca²⁺, whereas the SER is a high-affinity and low-capacity Ca²⁺ pool [40]. So, while the SER moves Ca²⁺ from the cytosol into the SER lumen under low cytosolic [Ca²⁺]_i, the mitochondria requires a more powerful stimulus, being necessary a local extra-mitochondrial Ca²⁺ concentration of approximately 0.5 mM, for neuronal mitochondria to take up Ca²⁺ actively [31, 41–43] (Figure 5). Disruption of Ca²⁺ regulation in either of these stores can compromise the neuronal function and survival [44] (Figure 5).

With particular emphasis in mitochondria, it is well recognized that at physiological concentration, Ca²⁺ is a powerful regulator of organelle metabolic activity, which acts primarily promoting ATP synthesis by stimulating crucial

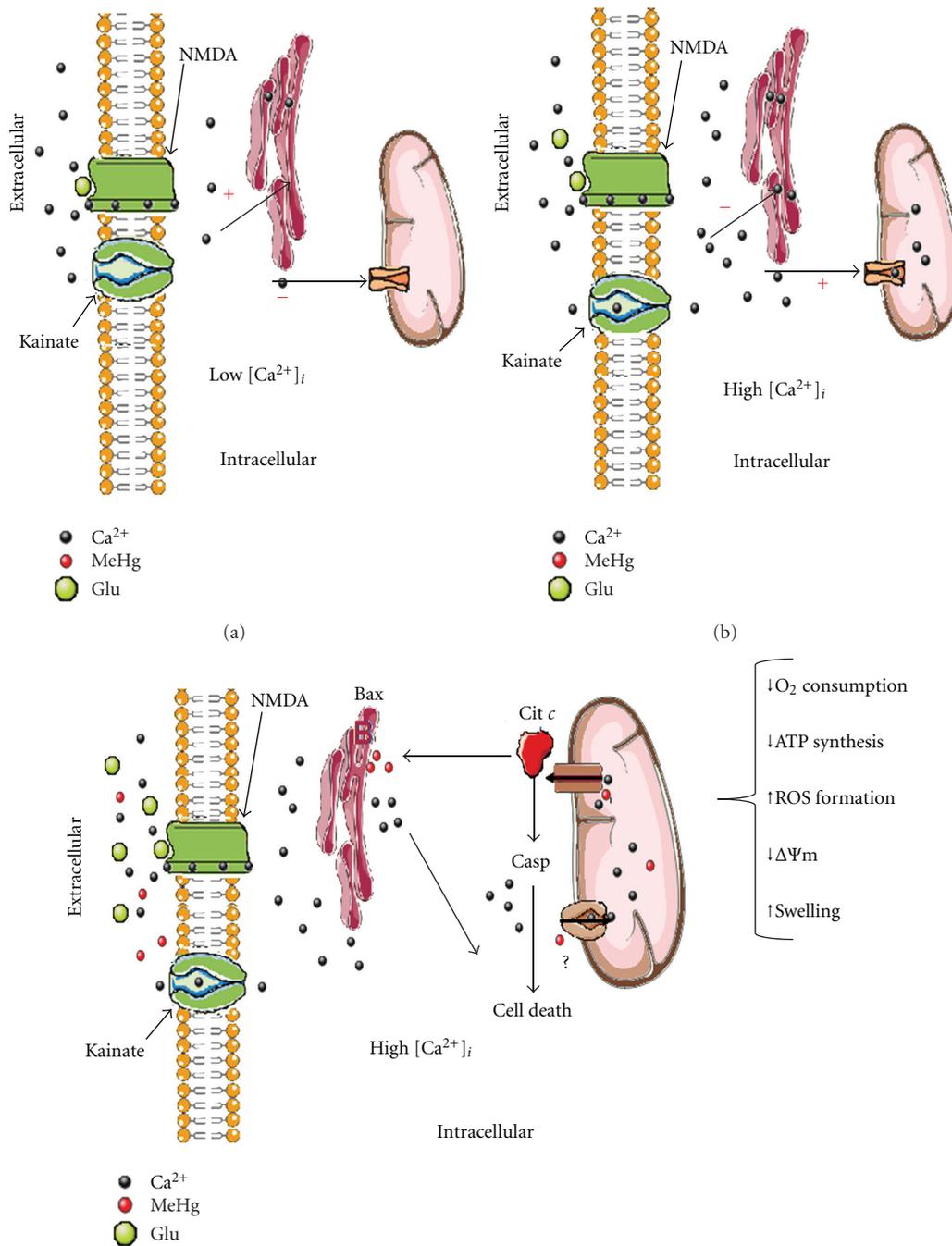


FIGURE 5: Schematic representation on the role of mitochondria and SER as intracellular Ca²⁺ stores and on the cellular death induced by MeHg via Ca²⁺ dyshomeostasis. (a) Under low cytosolic [Ca²⁺]_i the SER preferentially moves Ca²⁺ from cytosol due its high affinity and low capacity to stores Ca²⁺; whereas (b) the mitochondria by presenting low affinity and high capacity to stores Ca²⁺ moves it under high cytosolic [Ca²⁺]_i; (c) the disruption of Ca²⁺ regulation produced by MeHg in either of these stores can lead to release of neuronal proapoptotic factors that may trigger cell death pathways. The scheme presented here is merely representative, and the scale of the different cellular structures does not represent the real size.

enzymes of Krebs cycle (pyruvate, ketoglutarate, and isocitrate dehydrogenases). On the order hand, mitochondrial changes that occur in most instances of cell death (apoptosis and necrosis) require an elevated influx of Ca²⁺ into matrix [30–38] (Figure 5).

It has been suggested that the outer membrane voltage-dependent anion channel (VDAC), a ruthenium red (RuRed-) sensitive Ca²⁺ channel, serves to regulate Ca²⁺ entry to mito-chondrial intermembrane space (Figure 6). Furthermore, mitochondria take up a large quantity of

Ca^{2+} across the inner membrane mainly via the activity of the mitochondrial calcium uniporter channel (MCU) [45] (Figure 6). This uptake is driven by the membrane potential ($\Delta\Psi_m$), and consequently the net movement of charge due to Ca^{2+} uptake collapses $\Delta\Psi_m$ [45]. Although there is a general consensus that MCU is dominantly responsible for the Ca^{2+} influx into mitochondrial matrix, further studies have identified other pathways related to mitochondrial Ca^{2+} influx, including the mitochondrial ryanodine receptor (mRyR), mitochondrial uncoupling proteins, Letm1 ($\text{Ca}^{2+}/\text{H}^+$ antiporter), and rapid mode of mitochondrial (RaM) [45] (Figure 6). It is also important to highlight here the ability of mitochondria in releasing Ca^{2+} . Under normal physiological conditions, Ca^{2+} efflux from mitochondria is mediated primarily by mitochondrial $\text{Na}^+/\text{Ca}^{2+}$ exchanger. Indeed, Ca^{2+} efflux can also occur through uniporter channel if $\Delta\Psi_m$ is collapsed. Interestingly, another Ca^{2+} efflux pathway is the classical mitochondrial permeability transition pore (MPTP), which is a large voltage-dependent channel formed by a set of mitochondrial proteins located in the inner membrane, mitochondrial matrix as well as on the outer membrane (Figure 6). Existing predominantly in the closed position, the pore transient opening under normal conditions may serve as a physiological means of releasing excess of metabolites, inorganic anions, and ions, especially Ca^{2+} [46–50]. Indeed, MPTP permits the passage of several apoptotic mediators from mitochondria and is also a primary target of others, especially the Bcl-2 family proteins [51–55]. In this circumstances, it is important to emphasize the key role of mitochondrial Ca^{2+} overload and oxidative stress in MPTP opening.

There is a general conception that Ca^{2+} overload results in mitochondrial dysfunction, which trigger apoptosis by many different and connected pathways. In fact, a variety of molecular events involved in apoptotic stimulation focus on mitochondria. Mitochondrial changes as exacerbated ROS formation, dissipation of the $\Delta\Psi_m$, altered redox potential, MPTP opening, release of apoptotic mediators (cytochrome c, Apaf-1 and apoptosis inducing factor (AIF)), and participation of proapoptotic Bcl-2 family proteins are among the diverse signals associated to apoptosis induction by intrinsic or mitochondrial pathways [16, 51–55] (Figure 5). Indeed, ATP synthesis failure associated with mitochondrial dysfunctions can lead to abnormal cellular homeostasis causing swelling and cellular disruption, which eventually lead to necrotic death.

2.1. MeHg Disturbs Intracellular Calcium Homeostasis: Effects on Mitochondrial Function. A number of reports have indicated that MeHg, at low micromolar concentrations, disrupts Ca^{2+} homeostasis and causes elevations in $[\text{Ca}^{2+}]_i$ in different cells types, ranging from neurons and neuroblastoma cells to T lymphocytes and Purkinje cells [56–61]. The Ca^{2+} involvement on MeHg neurotoxicity is supported by several data, including the findings showing that the BAPTA (a Ca^{2+} chelator) is able to protect granule cells from MeHg-induced mortality after short periods of exposure. The regulation of other divalent cations besides Ca^{2+} , such as Zn^{2+} may be also

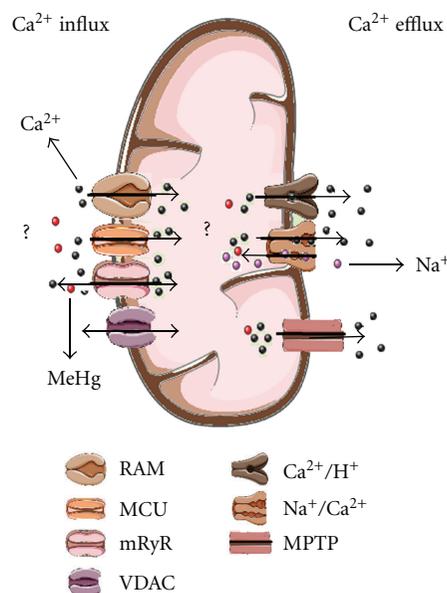


FIGURE 6: Representation of different channels responsible for Ca^{2+} influx/efflux in mitochondria and the possible interaction of MeHg with these channels. VDAC: outer membrane voltage-dependent anion channel; RAM: rapid uptake model; MCU: inner membrane calcium uniporter channel; mRyR: mitochondrial ryanodine receptor; $\text{Ca}^{2+}/\text{H}^+$ antiporter; $\text{Na}^+/\text{Ca}^{2+}$ exchanger; MPTP: mitochondrial permeability transition pore. The scale of structures represented in this scheme does not represent the real size.

disrupted during MeHg exposure; nevertheless, here we will focus on the effect of MeHg in Ca^{2+} homeostasis [62].

As previously mentioned, it has been postulated that the effects of MeHg on neuronal Ca^{2+} homeostasis, at least in part, involve the excitatory amino acids receptors (either NMDA and non-NMDA receptors), since that channel blockers as ω -conotoxin GVIA and nifedipine delay MeHg-induced elevation in $[\text{Ca}^{2+}]_i$ levels [19, 20]. In accordance, several studies have demonstrated that the glutamate overactivation of NMDA receptor induced by MeHg inhibition of glutamate uptake can raise intracellular Ca^{2+} influx and ROS overproduction [16]. In addition, experimental evidence also supports the participation of L- and N-type Ca^{2+} channels on MeHg neurotoxicity given that blockers of voltage-dependent Ca^{2+} channels prevent the appearance of neurological disorders in rats exposed to MeHg [63, 64]. In this sense, a recent work suggested that canonical transient receptor potential channels (TRPC), Ca^{2+} -permeable cationic channels, could be important for the mechanisms of cytotoxicity and neuronal impairment mediated by MeHg. In fact, in this study was observed that MeHg that potentially activates TRPC5 as well as TRPC4 channels via binding to the two extracellular cysteine residues near the channel pore, whereas silence of TRPC5 gene with small interfering RNA (siRNA) or blockade of TRPC channel activity with pharmacological tools alleviates MeHg-related cytotoxicity [65]. Notwithstanding, some findings have shown that MeHg toxicity may be triggered by elevating $[\text{Ca}^{2+}]_i$ through activation of phosphatidylcholine-specific phospholipase C (PC-PLC), which could contribute

to the entry of extracellular Ca^{2+} as well as to the opening of transmembrane Ca^{2+} channels from intracellular Ca^{2+} store [66, 67].

Numerous lines of evidence indicate that the MeHg-mediated neuronal cells death is associated with a severe loss of $[\text{Ca}^{2+}]_i$ homeostasis [58, 59, 68]. In neurons and NG108-15 neuroblastoma cells, MeHg causes a characteristic biphasic increase in $[\text{Ca}^{2+}]_i$ that consists of an initial release of Ca^{2+} from one or more intracellular stores into the cytosol (“first-phase”), and a secondary influx of $[\text{Ca}^{2+}]_e$ (“second-phase”) [56, 58]. However, it is necessary to consider that the elevation of $[\text{Ca}^{2+}]_i$ occurs at much lower MeHg concentrations in cerebellar granule cells than in NG108-15 cells, which could explain, at least in part, the different sensitivity of granule cells to the toxic effects of MeHg as compared to other neuronal cells [56, 58]. In analogy, there is evidence that elevated $[\text{Ca}^{2+}]_i$ has an important role in dimethylmercury (di-MeHg-) induced cell death. Of note, Ca^{2+} deposits (as calcospherites, a spherical mass of calcium salts and organic matter) were found in cerebellar slices of rats treated with di-MeHg. The cerebellar accumulation of calcospherites was directly proportional to exposure time, and the atrophy of the granule cell layer was evident at later time points [69]. Moreover, it has been observed that adjacent Purkinje cells did not show any sign of cell loss or death, nor accumulate Ca^{2+} [69]. These findings suggest that the presence or absence of Ca^{2+} deposits within the cerebellum seems to be directly correlated with sensitivity or resistance of cells to MeHg neurotoxicity. Noteworthy, it is important to mention here that this study was done with di-MeHg which is much more toxic to humans than mono-MeHg [68]. This is important to avoid confusion between mono-MeHg and di-MeHg, particularly in view of the extreme toxicological effects of di-MeHg to humans (even at low concentrations). Other important critical aspect here is the involvement of cerebellum in MeHg toxicity. Although this seems to be the case for humans (and cats), it is difficult to find in rodent literature “a clear picture” that mono-MeHg targets preferentially cerebellum than other brain structure. It is possible that cerebellum of rats be more sensitive to di-MeHg, which has been little used (possibly because of its chemical instability and toxicity).

Important early studies by Yoshino et al. [70] showed that MeHg is able to accumulate rapidly in mitochondria. In this way, subsequent works indicated a spectrum of mitochondrial effects, either directly or indirectly via Ca^{2+} overload, of MeHg both *in vivo* and *in vitro*, including alterations in complex III of the mitochondrial electron transport chain (ETC), depression of respiration and ATP production, swelling of the mitochondrial matrix, and loss of $\Delta\Psi_m$ with subsequent release of cytochrome c [71–80]. The loss of $\Delta\Psi_m$ seems to result from MPTP opening, which can be prevented by treatment with the pore-blocking agent cyclosporin A (CsA) or bongkreic acid [81–84]. In several studies CsA, but not FK506, that is similar to CsA but without MPTP inhibitory activity, provides a marked degree of neuroprotection against MeHg, supporting the involvement of MPTP in the MeHg neurotoxicity [73, 85–87]. Other experimental evidence shows that mitochondria contributes

to the MeHg-induced first-phase $[\text{Ca}^{2+}]_i$ increase and subsequent cell death through opening of the MPTP [56].

To note, mitochondria have been considered the principal source of intracellular Ca^{2+} release in culture of cerebellar granule cells during MeHg exposure [73]. It has been documented that MeHg affects mitochondrial Ca^{2+} ($[\text{Ca}^{2+}]_m$) regulation through at least two potential mechanisms. The first involves its direct interaction with mitochondrial proteins, which is thought to alter the ability of mitochondria in functioning normally. Corroborating this idea, there is evidence that MeHg inhibits mitochondrial nucleic acid synthesis and interferes with the electron transport chain via direct interaction with respiratory proteins [79] as well as causes loss of inner mitochondrial membrane potential in isolated mitochondria from different tissues, including neuronal cells [48, 71, 73, 85, 88, 89]. The second mechanism is associated with an indirect inhibition of mitochondrial function via excessive uptake of Ca^{2+} into the mitochondria. The elevated $[\text{Ca}^{2+}]_m$ may depolarize the inner mitochondrial membrane, inhibit the tricarboxylic acid cycle and the mitochondrial ATPase, dissipate the mitochondrial proton gradient, and promote MPTP opening [88–94]. Of particular importance, $[\text{Ca}^{2+}]_m$ can be increased after Ca^{2+} release from SER. Experiments in NG108-15 neuroblastoma cells and cerebellar granule cells show that MeHg causes Ca^{2+} release from SER, primarily through IP3 receptor, which is buffered by mitochondria (tentatively to maintain $[\text{Ca}^{2+}]_i$ homeostasis) [95, 96]. Corroborating these findings, an elegant study performed by Budd and Nicholls [97] demonstrated that mitochondria can store and release large amounts of Ca^{2+} under both physiological and pathological conditions in granule neurons [96–98]. Taken together, these data suggest that the excessive uptake of $[\text{Ca}^{2+}]_m$ after release of Ca^{2+} from the SER is a potential signal for the MPTP opening and $[\text{Ca}^{2+}]_m$ release from the mitochondrial lumen into the cytosol in cerebellar granule cells exposed to MeHg. These observations are also consistent with the hypothesis that both Ca^{2+} ionophore A23187 and MeHg cause a rapid and massive increase in mitochondrial Ca^{2+} influx, which secondarily triggers the overproduction of ROS that facilitates MPTP induction, loss of the electron transport chain activity, and the ensuing mitochondrial death [98]. Thus, an uncontrolled release of Ca^{2+} from the mitochondria may occur during oxidative stress, a condition resulting from the imbalance between the production of free radicals and the counteraction by the cellular antioxidant defenses [49, 99].

Based on the evidence addressed previously, mitochondria must be considered critical in the regulation of neuronal cell death induced by MeHg via Ca^{2+} dyshomeostasis and/or ROS generation (under oxidative stress conditions) [48–50]. In this sense, literature data have pointed that ROS formation is not the cause of mitochondrial dysregulations after MeHg exposure but rather a secondary event that reflects MeHg-induced elevations in mitochondrial Ca^{2+} levels [48, 53]. In agreement, an *in vitro* study revealed that the treatment of striatal synaptosomes with the antioxidant Trolox was effective in reducing ROS levels but failed on restoring mitochondrial damage induced by MeHg [100]. Similarly,

in a recent work using rat cortical slices was evidenced that the flavonoids quercitrin and quercetin reduced significantly mitochondria MeHg-generated ROS production, which was dependent upon an increase in intracellular Ca^{2+} levels [74, 101].

2.2. MeHg-Induced ROS Formation and Mitochondrial Dysfunction: Effect of Antioxidants. As already cited before, mitochondrial dysfunctions elicited by MeHg may lead to a mitochondrial burst of ROS production. ROS are important mediators of damage to cell structures, including lipids and membranes, as well as proteins and nucleic acids [102, 103]. In mitochondria, ROS may impair energy metabolism by inducing oxidative structural changes and the ensuing loss of activity in a number of mitochondrial enzymes that play critical roles in ATP production [42]. Besides, the direct action of ROS on mitochondrial membrane lipids and proteins results in the activation of apoptotic cascades by MPTP opening dependent or independent mechanisms [51, 53, 54].

The detrimental effects of ROS are balanced by the antioxidant action of nonenzymatic and enzymatic systems [104]. Indeed to ROS overproduction, *in vivo* and *in vitro* experimental observations have shown that the toxic effects of MeHg usually are accompanied by significant deficits in antioxidant defenses. MeHg can cause a decrease in the endogenous nonenzymatic antioxidants as well as an inhibition of the antioxidant enzymes [104–110]. Thus, it is reasonable to suppose that the cellular/mitochondrial ROS production may be directly or indirectly triggered by MeHg. In fact, the high affinity of MeHg by $-\text{SH}$ groups might decrease GSH content and consequently facilitates ROS formation [11]. On the other hand, MeHg also could exacerbate ROS formation by increasing $[\text{Ca}^{2+}]_i$. From a molecular point of view, as highlighted in Figure 7, these factors (GSH depletion, ROS and Ca^{2+}) exist as a pyramidal network, where they may act independently or through a complex interaction to activate the cascade of events involved in cell injury mediated by MeHg. With this in mind, the following sections discuss two possible antioxidant therapies (natural and synthetic) that could be effective in ameliorating the symptoms of MeHg intoxication by interfering with these factors.

2.2.1. Natural Antioxidants against MeHg-Induced Toxicity.

Despite the massive efforts in the search for new drugs that counteract mercurial toxicity, there are no effective treatments available that completely abolish its toxic effects. In general, the available antidotal strategies to treat mercury poisonings are largely based on chelating therapies. The use of sulfhydryl-enriched chelators is based on the high affinity of MeHg to $-\text{SH}$ groups, leading to mercury elimination from tissues predominantly via renal excretion. However, these drugs are of limited use, because of their adverse side effects [111]. Moreover, it has been proposed that chelating therapies are ineffective in poisonings with organic forms of mercury [112], although this issue remains controversial [113, 114]. Nevertheless, there is general agreement that metal chelators are unable to completely

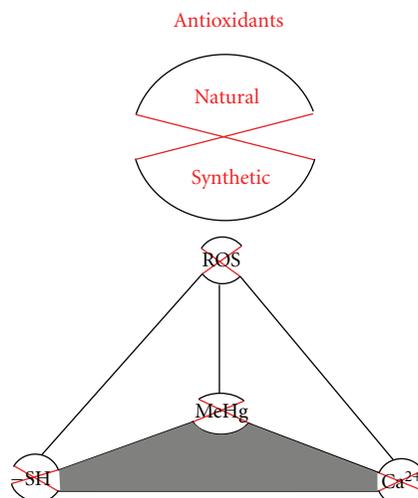


FIGURE 7: Pyramidal network involved in MeHg neurotoxicity: Arrows denote the interlinked possible pathways by which MeHg may cause cellular damage and the protective effect of natural and synthetic antioxidants against MeHg neurotoxicity by blocking oxidative events triggered via $-\text{SH}$ depletion, ROS formation, and Ca^{2+} dyshomeostasis. For full details, see the text.

eliminate mercurials' body burden and by inference, toxicity. Thus, the use of compounds with antioxidant properties and no apparent side effects could represent an efficient adjuvant strategy to counteract MeHg toxicity. Therefore, various purified phytochemicals or plant extracts have been shown to confer some protection against MeHg-mediated excitotoxicity and oxidative stress *in vivo* and *in vitro* [106, 115]. Possible mechanisms involved in the mitigation of MeHg effects by phytochemicals may include the reduction of ROS production, activation of enzymatic antioxidant systems, restoration of the mitochondrial membrane potential, and modulation of cell signaling pathways [116]. Dietary phytochemicals may also affect additional endpoints such as MeHg bioavailability and pharmacokinetics [117, 118]. In particular, natural products (such as flavonoids) whose antioxidant properties have been well described [119–121] could represent important therapeutic choices. In this sense, it has been characterized in both *in vitro* and *in vivo* experimental models that flavonoids exert beneficial effects by preventing or reducing free radical production, blocking Ca^{2+} influx into cells, chelating iron, and exerting anti-inflammatory action [100, 121, 122] (Figure 7). Indeed, such class of compounds has displayed neuroprotective effects in several experimental models of neurodegeneration [123–125]. Of particular significance, the current literature shows that plant extracts containing flavonoid provide protection against MeHg-induced neurotoxicity in mice [115, 121]. The protective effect of flavonoids has been correlated with their capacity in detoxifying the H_2O_2 generated in the presence of mercurials [71]. In addition to a direct interaction with H_2O_2 , it is possible that flavonoids may also reduce H_2O_2 production via inhibition of Ca^{2+} influx into cells or mitochondria [105] and/or by forming redox inactive complexes with iron, rendering this prooxidant unavailable for Fenton reaction.

A recent *in vitro* study using the flavonoids quercetin, rutin, and quercitrin showed that lipid peroxidation and ROS generation in both mitochondria-enriched fractions and cortical brain slices exposed to MeHg were significantly reduced by quercetin and quercitrin [74]. Likewise, further findings demonstrated that mangiferin was effective in offering protection to human neuroblastoma cells against the toxic effects of MeHg. The protective effect of this flavonoid was attributed to its antigenotoxic, antiapoptotic and antilipid peroxidative potential plausibly because of its free radical scavenging ability, which reduced the oxidative stress and in turn facilitated the downregulation of mitochondrial apoptotic signalling pathways [126–128]. Corroborating these experimental observations, a current study performed by Franco et al. showed that the flavonoids myricetin, myricitrin, and rutin are able in reducing mitochondrial dysfunctions in mouse brain mitochondrial-enriched fractions treated with MeHg *in vitro*; being that the myricetin displayed higher protective effect against MeHg-induced mitochondrial toxicity when compared to myricitrin and rutin [129]. Additionally, only the myricetin was able to inhibit completely ROS formation and lipid peroxidation MeHg induced [129]. The scavenger property of this flavonoid was considered, at least in part, responsible for its protective effects against mitochondrial dysfunction induced by MeHg.

From a molecular point of view, the antioxidant activities of flavonoids can be influenced by their chemical structure [130, 131], and there are several molecular characteristics that confer the ability of a given flavonoid to promptly donate electrons and reduce reactive species. Basically, polyphenolic flavonoids possess a diphenylpropane (C6C3C6) skeleton [130, 131]. The presence of hydroxyl groups linked to phenolic rings correlates with their capability to donate electrons [132]. The positions and, more importantly, the amounts of hydroxyl groups present in the polyphenolic skeleton increase their ability to neutralize reactive species [130, 131]. The removal of this functional group from flavonoids has been reported to impair their antioxidant potency [130–132]. Lack of saturation at the C-ring is another structural property that confers antioxidant ability to flavonoids. Similarly, the blockade of the hydroxyl group in the C-ring through glycosylation has also been reported to decrease the antioxidant ability of this class of compounds [130–133].

Despite the potential use of natural products (specially flavonoids) to counteract the MeHg-induced cell damage via mitochondria-mediated ROS generation, more *in vivo* experiments are needed to validate the efficacy of flavonoids in attenuating MeHg-mediated injuries, particularly in view of the recently published synergistic neurotoxic action of quercetin and MeHg in adult mice [101].

2.3. Synthetic Antioxidants against MeHg-Induced Toxicity. Similar to natural compounds, numerous synthetic compounds exhibit potent antioxidant properties that could be effective in reducing the oxidative damage elicited by MeHg. However, the focus of this section will be in some selenium (Se) compounds, since it has long been hypothesized that

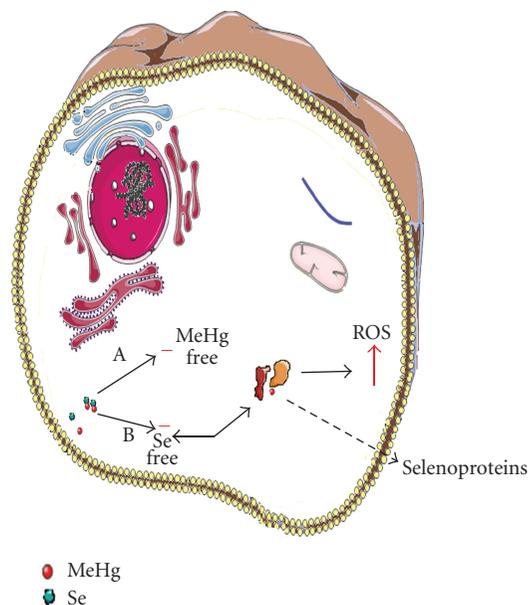


FIGURE 8: Possible role of Selenium (Se) on MeHg toxicity: (A) MeHg toxicity reduced by formation of complex MeHg-Se; (B) MeHg toxicity increased due Se depletion by formation of complex MeHg-Se: ↓ Selenoprotein activities and synthesis and ↑ ROS.

Se may protect against the harmful effects of mercury, particularly organic MeHg [134].

Most of beneficial effects exhibited by Se in biological systems are connected to activity of enzymes glutathione peroxidases family (GPx1–4), thioredoxin reductase family (TrxR1-2), thioredoxin glutathione reductase (TGR), and iodothyronine deiodinase isoforms (DIO1–3), which present this element as a structural component [135].

Regarding to mercury toxicity, it has been pointed that the strong interaction between mercury and Se is an important mechanism related to neuroprotection offered by selenium compounds [135, 136] (Figure 8). From this point of view, the Se's protective role is associated with the formation of stable complex(es) resulting of the reaction between mercurials and selenohydril/selenol groups (–SeH) formed from de selenium compounds. However, it is important to mention here that little is known about the toxicokinetics of these complexes in biological systems. Furthermore, some studies argue that Se sequestration due to mercury binding to Se could compromise Se's biological functions and availability, mainly by impairing selenoenzyme activities and synthesis [135] (Figure 8).

The use of synthetic compounds containing selenium has received growing interest as therapeutic strategies in the treatment of mercury intoxication symptoms, including the detrimental effects of MeHg in CNS [135–137]. Of particular importance are the GPx- and TRxR-like activities displayed by ebselen and diphenyl diselenide, two simple synthetic organoselenium compounds that have been considered potent antioxidant and neuroprotective agents in different experimental models [136, 138]. Current literature findings show that these compounds may be reduced by mammalian

(TrxR) forming selenolate intermediates that are potent nucleophiles and can readily react with electrophilic species, including ROS [138]. This implies that these compounds could efficiently attenuate the oxidative damage caused by MeHg. Corroborating this hypothesis, a recent study revealed that diphenyl diselenide reduced cerebral oxidative stress as well as Hg deposition in the liver, kidneys, and brain of adult mice exposed to MeHg [13]. In this work the authors assume that the effectiveness exhibited by compound is derived from the formation of a stable complex (MeHg-Selenol) due interaction of MeHg with a “selenol intermediate” [13].

Based on the observations previously cited, it is reasonable to suppose that ebselen and diphenyl diselenide might diminish the neurotoxic action of MeHg by increasing the excretion of MeHg from the body due their abilities in forming inert complexes with MeHg [13]. Regarding to the involvement of glutamatergic system on MeHg neurotoxicity, there is *in vitro* evidence that both organoselenium compounds as well as MK-801 were effective on blocking ROS formation caused by MeHg in cortical rat brain slices by maintaining the hydrogen peroxide status at low physiological levels and/or through a direct modulation of the NMDA receptor redox site [110]. Likewise, the treatment of cerebral cortex slices from young rats with ebselen restored the glutamate uptake and cell viability changed by MeHg exposure [139]. Differently, in this experimental protocol diphenyl diselenide did not modify these parameters [139].

In addition, data from *ex vivo* experiments show that glutamate release from brain synaptosomal preparations and glutamate uptake by brain cortical slices is increased in rat pups exposed to MeHg during the suckling period and that these effects were prevented by ebselen [140]. Equally important, there is evidence that ebselen was effective in reversing the inhibition induced by oral exposure to MeHg on glutamate uptake of brain cortical slices of adult mice as well as in restoring the activity of enzymes glutathione peroxidase and catalase activity, which were modified by MeHg. These protective effects of ebselen were related to its ability to detoxify H₂O₂ [140].

Of particular importance, recently an elegant study demonstrated the potential ability of ebselen and diphenyl diselenide in preventing neuronal dysfunction caused by MeHg via cytoskeletal proteins disruption. In this work MeHg exposure induced hyperphosphorylation of the high molecular weight neurofilaments subunit from slices of cerebral cortex of young rats, and both selenium compounds ebselen and diphenyl diselenide were efficient in reversing these alterations [141].

As stated before, there is numerous experimental evidence suggesting that MeHg exposure may induce damage in mitochondria from different organs and tissue [74], and that this effect could be associated to ROS overproduction and/or increased intracellular calcium levels [74, 142]. However, to the best of our knowledge, there are no reports on the role of organoselenium compounds in isolated mitochondria challenged by MeHg. Thus, although the organic selenium forms cited here may represent an interesting class of compounds as therapeutic agents against MeHg intoxication,

further research to evaluate the efficacy and safety of such compounds will be needed.

3. Conclusion

Despite the large number of studies on MeHg toxicity, the understanding about the metabolism and toxicokinetics of MeHg as well as the molecular mechanisms involved in its neurotoxic effects remains to be fully elucidated. Meanwhile, taken together the data discussed in this paper collaborate for a better understanding of the multifactorial mechanisms (glutamate and calcium dyshomeostasis, as well as mitochondrial dysfunction) involved on neurotoxicity produced by MeHg and point the possible interlinked pathways by which MeHg might cause neuronal damage, with special emphasis on mitochondrial disturbances, since the mitochondria seem to be a key organelle implicated in the detrimental effects triggered by MeHg both *in vivo* and *in vitro*. In this regard, experimental studies show that both synthetic and natural antioxidants may be considered promising molecules in counteracting the toxic effects elicited by MeHg. Thus, although experimental research has been highly instrumental in shedding novel information on aspects involved in MeHg-induced neurotoxicity, further studies on the precise temporal relationship between the listed phenomena, as well as potential therapeutic/antidotal strategies to counteract the mitochondrial dysfunction produced by MeHg are warranted.

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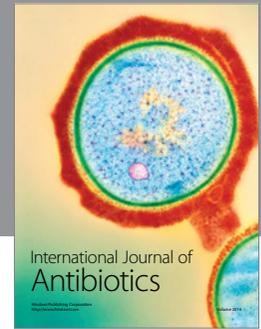
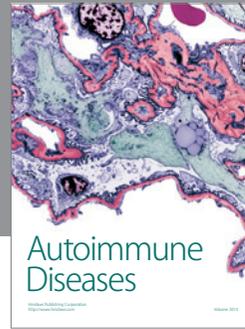
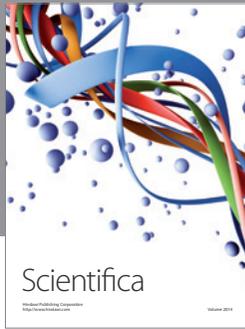
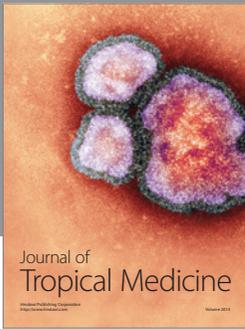
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