Review Paper:

Heavy Metals Effect on Mitochondrial Oxidative Phosphorylation, Oxidative Stress and Apoptosis

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Abstract

Heavy metal pollution has become a classic problem until today, especially in urban areas of developing countries. In developed countries, heavy metal pollution has begun to decrease since the last hundred years. The heavy metals in environment come from industrial products and waste, mining and smelting, vehicle emissions, agriculture etc. Heavy metal pollutes the air, soil, water and food from agricultural and animal products, thus poisoning living things both humans, animals and plants. Heavy metals enter the human and animals through contaminated air and ingestion of contaminated food and drink. Heavy metal could induce neurodegenerative damage, cancer, anemia, acute and chronic toxicity. Heavy metals are distributed to several organs such as the brain, liver, kidneys, skeletal muscle and heart etc.

Several studies have proven mitochondria as targets of heavy metal poisoning. Heavy metals interfere with energy formation in the mitochondria, induce oxidative stress, apoptosis and mitophagy. The main objective of this study was to identify the influence of mercury, cadmium, arsenic and lead on mitochondrial oxidative phosphorylation, oxidative stress and mitochondria-mediated apoptosis. In this review, we will discuss the toxicity mechanism, the factors that cause toxicity and their toxicity effects on the mitochondria and cells.

Keywords: Apoptosis, Heavy Metals, Mitochondrial Antioxidant, Mitochondrial-Mediated Oxidative Stress, Oxidative Phosphorylation.

Introduction

Heavy metals have been used for a long time and are still increasing in some countries, especially in developing countries[1]. Heavy metals in the environment come from mining and smelting, industrial products and waste, vehicle emissions, burning of fossil fuels and pesticides[2–4]. The heavy metals pollutants are contaminating soil, water, air and food from agricultural and animal products. Thus, heavy metals can enter the human body through breathing and contaminated food or water.‘

There are two kinds of heavy metals, essential and nonessential heavy metals. Various physiological and biochemical function in the human body requires essential heavy metals such as iron (Fe), zinc (Zn), copper (Cu), cobalt (Co), magnesium (Mg), manganese (Mn) and selenium (Se)[5]. For example, Fe is needed for hemoglobin production. Although essential heavy metals are beneficial to the body, if the level exceeds its normal value, it will disrupt the body’s function or will cause a disease /toxicity[1]. For example, high levels of Fe post-transfusion in thalassemia patients cause cardiac ciderosis, left ventricular heart failure, liver fibrosis, cirrhosis and hypothyroidism[6].

Meanwhile, non-essential metals have no biological functions such as arsenic (As), mercury (Hg), lead (Pb), cadmium (Cd), nickel (Ni), chromium (Cr), barium (Ba) etc[5]. These metals cannot be degraded and their accumulations in the food chain induce human health risks[4]. There are top five heavy metals with high degree toxicity that induce multiple organ damage, even at a low level of exposure. They are arsenic, mercury, lead, chromium and cadmium. Arsenic, mercury, lead and cadmium are commonly found in the environment and have been suggested as being very toxic and carcinogenic.

Epidemiological and experimental studies showed a correlation between exposure to these metals and the incidence of cancer in humans and animals. The United States Environmental Protection Agency (US EPA) and the International Agency for Research on Cancer (IARC) classify these metals as human carcinogens B[5]. The toxicity of each metal depends on many factors including duration of exposure, quantity, method of exposure and chemical form of heavy metals[4,7].

Heavy metals were accumulated and distributed to mitochondria, especially in the organs with high energy demand such as the brain, liver, kidneys, bones and heart muscle[8–11]. They induce various adverse effects in the mitochondria. Although it is well known that mitochondria are targets for heavy metal toxicity, nevertheless, the

mechanisms of disturbance of mitochondrial function by heavy metals are not well understood.[12] Various studies showed that heavy metal increases the generation of reactive oxygen species (ROS)[13–15] which causes various pathological conditions such as toxicity, cancer, neurotoxicity and genotoxicity[3–5]. These findings indicate that heavy metal mediated toxicity is associated with mitochondrial disruption.

Mitochondria are essential as a fuel generator or bioenergetics and have other functions such as cell death signaling and regulation of oxidative stress[16]. As bioenergetics, mitochondria regulate glycolysis, Krebs's cycle and oxidative phosphorylation (OXPHOS), OXPHOS produces free radical or reactive oxygen species (ROS) which is oxidized by antioxidant enzymes within the mitochondria. Mitochondria alter to apoptotic process after receiving a cell death signal or after disruption of the mitochondrial membrane, which releases cytochrome c (cyt c). Heavy metals enter the cell membrane via the calcium channel (e.g. the receptor in a neuron is N-methyl D-aspartate /NMDA receptor)[8,17].

Then, the heavy metals enter the mitochondria via the calcium transporters because of their cation molecular mimics with calcium and enter the inner mitochondrial membrane by passive diffusion because of the alkaline mitochondrial pH and negative mitochondrial matrix charge (heavy metals are positive charge)[18].

Mitochondria as the target of heavy metals is poisoning through disruption of energy synthesis, induction of oxidative stress, alteration of mitochondrial membrane potential (MMP) and mitochondrial permeability transition pore (MPTP) which induces apoptosis or necrosis[3,19–26]. Insufficient ATP disrupts the chemical reaction process that requires ATP. An oxidative stress condition with a high level of ROS can cause damage to the membranes, proteins and nucleic acids. Normally, the body maintains a dynamic balance of apoptosis. Imbalance, both inhibition and excessive apoptosis, are harmful to the cells. Inhibition of apoptosis occurs when toxicants or free radicals are increasing which will promote the induction of tumorigenesis. On the other hand, an increase in excessive apoptosis due to heavy metals poisoning can induce degeneration such as neurodegeneration[3,5].

This review highlight the impact of mercury, lead, arsenic and cadmium (MLAC) on the mitochondria, especially on OXPHOS, oxidative stress and mitochondrial-mediated apoptosis. Several studies have shown the effect of these metals on mitochondria. For example, these metals could inhibit OXPHOS by decreasing complex I, II, IV[8,15,20,27], enhance oxidative stress by increasing the ROS production and decreasing the mitochondrial antioxidant[13–15]. ROS can induce DNA damage, protein oxidation and lipid peroxidation, which is associated with the onset of various diseases[2]. These metals also induce mitochondrial-mediated apoptosis through several pathways; the alteration of the MMP and MPTP and decrease bcl2 (anti-apoptotic protein level)[14,28]. Heavy metals toxicities vary depending on the chemical form, route and duration of exposure[29].

Heavy Metals and Impairment of Mitochondrial Oxidation Phosphorylation: Energy is a result of the four-state respiration process: the conversion of glucose into pyruvates, glycolysis, Krebs cycle and oxidative phosphorylation. The electrons pass through a series of proteins through an oxidation-reduction reaction called electron transport chain (ETC) process.

In glycolysis, several studies have shown that heavy metals can inhibit the activity of glycolytic enzymes. Ramirez-Bajo et al[4] have shown that Hg and Cd inhibit the activity of the hexokinase and phosphofructokinase enzymes but do not change glucose-6-phosphate and fructose-6-phosphate levels. The mechanism of toxicity of these heavy metals to glycolytic enzymes is the interaction between metals and proteins in the cysteine SH group because of their high affinity for cysteine while these groups determine the structure and conformation of the active site of an enzyme in its catalytic function.

Bansal et al[30] had reported that heavy metals affect the tricarboxylic acid (TCA) cycle or the Krebs cycle enzyme. They showed that Pb, Cd and Pb+Cd suppressed the activity of isocitrate dehydrogenase, succinate dehydrogenase, α-ketoglutarate dehydrogenase and malate dehydrogenase. Pb and Cd can reduce glycolytic enzyme activity by up to 30% and this metal combination has more or fewer effects. There are five protein complexes involved in OXPHOS; NADH dehydrogenase (complex 1), succinate dehydrogenase (complex 2), cytochrome C reductase/COX BC (complex 3), cytochrome c oxidase /COX C (complex 4) and ATP synthase (complex 5).

Various studies have shown that these OXPHOS protein complexes are included as the target of heavy metals toxicity. Some studies show that arsenic inhibits rat brain energies formed by inhibiting activities of complex I, II and IV and reduce ATP synthesis.[14,15] Mercury inhibits state 3, decreases complex 1,2,3,4 activity and decreases ATP synthesis[12,20,32]. Cadmium decreases COXII and COXIV and inhibits FCPP uncoupled cell respiration[12,21,33], but Chang et al[35] found that cadmium increases COX in pancreatic β cell. Meanwhile, lead inhibits complex III (table 1).[36]

The mechanism of disturbance of mitochondrial function including OXPHOS by heavy metals, is not well understood[12]. However, heavy metals have a high affinity for sulfhydryl (-SH) groups[37]. Several non-essential heavy metals directly bind to sulfhydryl groups at the active site of most enzymes and inactivate them[38].

Heavy metals toxicity is associated with their interaction with metalloenzymes. They substitute the essential metal

required (e.g. Zn, Cu and other trace metal) from the natural binding site. The results are inactivation or reduction of the metalloenzymes effectiveness[37–39].

Heavy metals increase the generation of ROS[14]. Mitochondrial DNA (mtDNA) is close to the inner mitochondrial membrane (IMM) and very susceptible to oxidative damage besides lack of histone and other related proteins, absence of introns and high levels of transcription in the coding region[40,41].

Table 1
A Brief Literature Survey Of The Impact Of Non-Essential Heavy Metals To The Mitochondria

Mitochondria	Dosage	Duration	Main Finding
MDA-MB231 breast cancer cell line	5 and 50 µM/ml CdCl2 solution	24 and 96 hours	Downregulation COX II and IV Increase ROS generation[21]
Pancreatic b-Cells	5 and 50 µM/ml CdCl2 solution	4, 8, 24 hours	Increase ROS generation Induce the loss of MMP Increase cyt c release Decrease bcl2 expression Increase 53 expression, PARP cleavage and caspase cascade[35]
Human embryonic kidney cell	CdCl 1.5 and 50 µM	2 minutes	Increase in MPT Loss of MMP Inhibit activities of ATPase, LDH, SOD and GPx Increase ROS content and LPO[7]
Rat	Inj Cd 20µmol/kg BW/day	16 days	Loss of MMP Increase ROS production Inhibition of GPx activity Elevation of GSH and MDA content Increase of DNA-SSB level[47]
HL-60 cell	25, 50, 100 µM Cd	18hours	Increase of cyt c release[61]
ZC-7901 grass cap cell line	18 nM Cd	0.5-10 hours	Decrease MMP Increase ROS level Decrease SOD and CAT level[22]
Zebrafish muscle and brain	Food containing 13 µg MeHg	49 days	On muscle: decrease state 3 and 4 respiration, decrease COX activity, decrease ATP production Brain (higher Hg concentration): have no significant change.[25]
Skeletal muscle zebrafish	13 µg MeHg contaminating food	25 and 49 days	Decrease state 3 Decrease ATP release Decrease COX IV protein level and inhibit COX IV activity Decrease cytb expression[20]
Wistar rat liver	5, 10, 15, 20, 40 µM/ml Hg2+ solution.	30 minutes	Decrease MMP Increase ROS level Inhibit state 3 and state 4 respiration Decrease mitochondrial ATP level Induce MPTP opening Increase cyt c release[27]
Human peripheral blood mononuclear cells (HPMC)	MeHgCl 0-5 µM	2-16 hours	Decrease GSH Increase cyt c Decrease bcl2[28]
	HgCl2 0-50 µM	8hours	No effect to cyt c No effect to bcl2

Skeletal muscle of chicken	25 mg/kg BW As2O3	12 weeks	Decrease of SOD and GSH[48]
Rats	Sodium arsenite 25ppm Waterborne	12 weeks	Decrease complex I, II, IV activities. Increase ROS, oxidation of protein and LPO Decrease MnSOD activity[15]
Isolated Liver Mitochondria	(50, 100, 200 µM) Arsenic III solution	30, 60 second	Increase ROS Decrease complex I and II activity Increase MDA Decrease GSH Decrease ATP content and ATP/ADP ratio[51]
CHOK1 cell, human-hamster hybrid cells	0.25, 0.5 and 1 µg/ml Arsenic solution	16, 30, 60 days	Increase citrate synthase Decrease COX[31]
Rat brain's	100ppm sodium arsenite	21, 28 and 3 months	Decrease SOD, CAT, GPx, GST and GR activity. Increase LPO, caspase 3 and 9 expression[13]
Rat brain's	2.5mg/kg BW sodium arsenite	4 weeks	Decrease complex I, II and IV activity Decrease ATP generation Decrease MnSOD activity Increase cyt c expression in the cytosol Increase bax/ bcl2 ratio Increase caspase 3 activity[14]
Yeast	1,000 µmol/l Pb	3 hours	Increase ROS Mutation to mtDNA[60]
Rat mitochondria	Pb^{2+} (10-160µM)	15 minutes	Increase ROS production Inhibit complex III activity. Increase mitochondrial membrane LPO Decrease ATP and GSH level Induce MPTP opening MMP collaps Increase cyt c release[36]

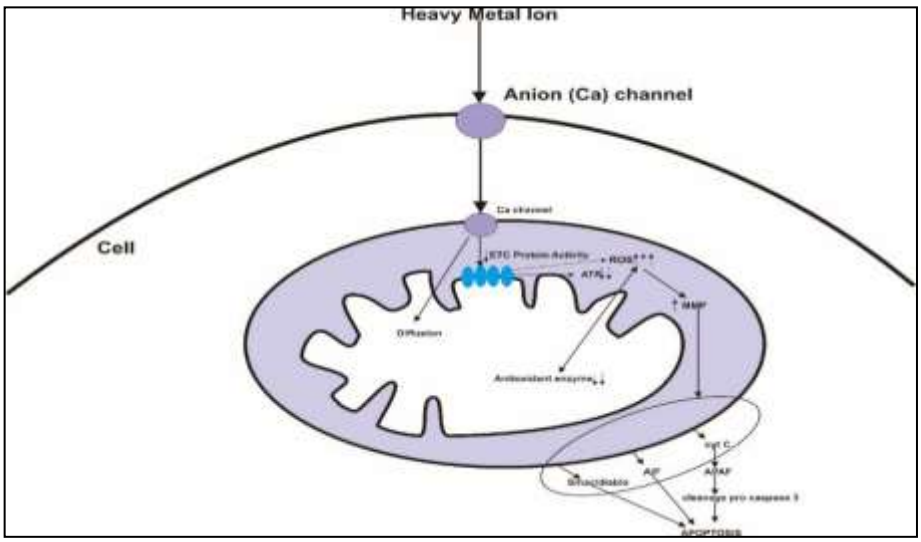

Figure 1: The Schematic Mechanisme of Heavy Metal Toxicity to the Mitochondria[36,51]. Heavy metals can enter cells through a transporter membrane or passive diffusion. heavy metals enter the mitochondria through the Ca channel and enter the mitochondrial matrix by diffusion. Heavy metals alter ETC pratein activity therefore decrease ATP production and increase ROS generation. ROS decrease antioxidant level and heavy metal directly inhibit antioxidant activity. ROS increase MMP and causes the release of pro-apoptotic proteins such as cyt c, AIF and smac/diablo.

Damage to mtDNA affects the transcription of the OXPHOS protein and alters the OXPHOS reaction[41]. ROS also may induce incorrect protein folding, induces protein aggregation and changes in protein conformation[42]. Modifying the structure of proteins causes cellular dysfunction[43] including enzymes used in the bioenergy process. Inhibition of the protein complex activity can reduce energy production which is important for cell viability and cell function.

Heavy Metals and Oxidative Stress: Oxidative stress refers to the imbalance between ROS generation and the antioxidant capacity[44,45]. ROS is mostly produced during the metabolic process. In eukaryotic organisms, mitochondria are the primary sites for the production O_2^- (superoxide) and H_2O_2 hydrogen peroxide),1-5% O_2 converting to $O_2^{\cdot-}$ during normal metabolism. Agents that block ETC in every step stimulate superoxide production[46]. The electron transport chain process produces H_2O and reactive oxygen species (ROS), superoxide, hydrogen peroxide and hydroxyl. Electron leakage from complex I and complex III causes oxygen to become ROS because oxygen has the ability to un-pair, which is unstable and highly reactive leading to the formation of ROS. ROS is neutralized by endogenous antioxidants such as glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione transferase (GST)[7,13–15,22,47,48].

In several conditions such as hypoxia, inflammation, radiation and xenobiotic (drug, metal toxicity, other chemical compounds), mitochondria produced more ROS compared to the normal condition[14,44,45,49,50]. Heavy Metals induces oxidative stress by increasing ROS production[14,27,33,35,36,51]. The mechanisms for increasing ROS generation by heavy metal are not as clear and easily understood as the mechanism for redox-active metal (Zn, Cu, Fe, Mn) and Fenton like reaction[46]. Therefore this review will try to explain the ROS generation mechanism in each heavy metal. Lead, mercury, arsenic and cadmium, decrease antioxidant activity because they have electron sharing affinities that can result in the formation of covalent attachment mainly between sulfhydryl groups of protein[46]. Most of the enzymes contain sulfhydryl groups at their active site where several non-essential heavy metals directly bind to it and inactivate the enzyme activity[38].

The toxicity of these heavy metals also is associated with its interaction with metalloenzymes. They replace the essential metal required from their natural binding site and inactivate these metalloenzymes[38,39,46]. Zinc is a cofactor of many enzymes. Superoxide dismutase (SOD), a metalloenzyme, requires a cofactor such as Mn^{2+}, Cu^{2+} and Zn^{2+} for their activity. Kumar et al[39] have shown that arsenic and mercury decreased SOD activity, but lead does not affect SOD activity.

Heavy Metals induces Cell Death: Programmed cell death or apoptosis is a process in which cell death is initiated through the activation of various apoptotic pathways. Apoptosis is an essential process for development, morphogenesis, immune regulation and tissue remodeling, but apoptosis can occur as a pathological reaction[52]. The cell death signal through apoptosis has two categories: receptor-mediated/extrinsic apoptosis and mitochondria-mediated/ intrinsic apoptosis.

Normally pro-apoptotic protein such as cytochrome c, the second mitochondrial activator of caspases (SMAC), direct inhibitors of apoptosis binding protein with low pI (diablo), serine protease high-temperature protein A2 (HtrA2), apoptosis-inducing factor (AIF) and endonuclease G (Endo G) are keeping in the mitochondria. The disruption of the mitochondrial membrane (disruption MMP or opening MPTP) can cause these proteins leakage and induce apoptosis. As known, heavy metals increase ROS, thereby disrupt MMP and open the MPTP. Heavy metal also decreases bcl2,[14] hence induce the formation of pore on the mitochondrial membrane which causes the release of pro-apoptotic proteins to induce apoptosis. The other conditions which induce MPTP opening are calcium overload.[12] Hg could induce Ca^{2+} intracellular overload.

Mitochondria and Cadmium: The most abundant and toxic heavy metal is cadmium[43]. Some industrial processes such as metal coating, cadmium batteries, pigments, plastics, tobacco and others are associated with Cd. The half-life of a Cd is very long, 17-30 years because of its low excretion ratio and its accumulation in organisms being the main causes of this Cd long half-life[43,46]. The liver and kidneys are the main organs of Cd accumulation[43,46]. Cd causes irreversible kidney tubular injury, osteoporosis, anemia, eosinophilia, non-hypertrophic emphysema and chronic rhinitis. Cd is banned and classified by the International Agency for Research on US Cancer and the European Union Restrictions of Hazardous Substances as potential carcinogens in humans because they can cause lung, prostate, pancreatic and kidney cancers.

Some cadmium properties become a toxicity factor to the cells. Cd is chemically similar to zinc [43] where zinc is one of the main metalloenzyme components, thereby Cd can replace zinc in biological system like enzyme[53]. Cadmium can bind ten times more strongly than zinc in certain biological systems and is difficult to remove[53]. Cd is a Ca agonist because of its proximity to the ionic ion crystal radius (Cd-0.097nm; Ca-0.099nm)[12]. Cd also has the ability as a dithiol reagent which can be involved in organic and inorganic synthesis, therefore Cd could disrupt biological synthesis[12,43].
Cannino et al[33] showed Cd has downregulation COX-II and IV in the breast cancer cell line (MDA-MB231). Mao et al[7] expose human embryonic kidney cells (HEK293 cell) to Cd and show that Cd inhibits respiration by inhibiting ATPase[7].

Cd induces oxidative stress, but the exact mechanism of their pathogenicity is not known[53]. Various reports indicating that

Cd induces ROS generation and decreases the antioxidant enzyme[7,35]. The mechanism that Cd induces ROS generation is still not clear, but Cd inhibits complex III of ETC that is one of causes of ROS generation (superoxide) induced by Cd[53]. Several studies showed that Cd alters GSH level, for example, injection 20µmol/kg BW/day Cd for 16 days increased tissue GSH levels in rat[47]. In other study, 7.25-116mg/L Cd is exposed to freshwater crabs for 48hours and result is decreasing on hepatopancreatic GSH content[54].

These result suggested that low level of Cd activated GSH synthesis against ROS and higher Cd exposure decreased GSH synthesis[54]. GSH protect the cell against oxidative stress and alteration of GSH level (either an increase or a decrease) indicates an oxidant status disturbances. When cells are oxidative challenged, GSH synthesis increases. As oxidative stress continues, GSH synthesis cannot efficiently supply the demand, therefore GSH depletion occurs[46]. Mao et al[7] showed that Cd evoked oxidative stress by inhibiting the antioxidant activity of lactate dehydrogenase (LDH), SOD and GPx as well as enhanced ROS level. Other studies have shown that Cd decreases the SOD and CAT activities.

Cadmium induces apoptosis by opening the MTP pore[47,55]. Cd can bind to sulfhydryl groups of mitochondrial membrane proteins, thereby induces MPTP opening which decreases MMP and releases apoptogenic proteins such as cyt c, AIF and smac/diablo, thereby induces apoptosis cascade[47]. The collapse of MMP is a critical step that occurs in all types of cells undergoing apoptosis[47]. Cd also increases several pro-apoptotic proteins and decreases several anti-apoptotic protein as is shown by Chang et al.[35] Cd decreased bcl2 expression, increased p53 expression, increased poly ADP-ribose polymerase (PARP) cleavage and caspase cascade, but no change in bax expression.

Mitochondria and Mercury: Mercury exists in metallic, inorganic and organic form. All form of Hg can be toxic and the toxic effects vary depending on the dose, chemical form, duration and level of exposure. Methylmercury is the organic mercury, the most common in the environment and it is formed when Hg+ or Hg^{2+} is methylated by activities of microorganisms in aquatic milieu. MeHg can bioaccumulate in fish and sea mammals through the aquatic food chain[17,56]. Methylmercury is lipid soluble, hence can easily cross both the blood-brain and placental barriers. Once absorbed, Hg has low excretion rate and accumulates in the kidneys, neurological tissue and the liver exhibits toxic effect including nephrotoxicity, neurotoxicity and gastrointestinal toxicity[46].

Outbreak environmental disasters because of MeHg in Japan (1950) and Iraq (1970) due to consuming MeHg contaminated fish represent that the brain is the main target organ for its toxicity with neurological symptoms such as cerebellar ataxia, paraesthesia, memory impairment and sensory disorders.[17] Among the various mechanisms suggested, no single mechanism explains all of Hg toxicity-phatological outcomes[46]. Several pathological mechanisms of Hg are related to the ability of Hg to bind to sulfhydryl groups, disorders of glutamate (glu) and calcium homeostasis and induce ROS generation. Similar to cadmium, Hg also has chemical similarities with zinc[43] and has high affinity for sulfhydryl groups, thereby Hg can replace essential metals (Zn) and binds to proteins that contain the -SH group reducing the effectiveness of these metalloenzymes or their function[17,37–39]. Methylmercury toxicities are associated with glutamate (glu) homeostasis disruption followed by loss of intracellular calcium homeostasis (intracellular Ca^{2+} overload)[17,29].

Methylmercury inhibits the absorption of glutamate (glu) and stimulates the release of cytosolic glu which causes an increase in glu levels in extracellular fluid. Glutamate overactivated the N-methyl-D-aspartate (NMDA) receptor in the brain thereby raising intracellular Ca^{2+} influx and ROS overproduction[17,29]. The elevation of intracellular Ca^{2+} level induces Ca influx to the mitochondria and causes mitochondrial disruption i.e. failure of energy metabolism, alteration in ETC complex activity, opening of MPTP, dissipation of MMP and ROS overproduction[17].

On other hand, calcium at high levels acts as a second messenger and alters protein phosphorylation, which then disrupts protein/enzyme function[29]. MeHg inhibits oxidation phosphorylation by decreasing inhibited complex 3 and complex 4, thereby there is decrease in rate of ATP release from skinned muscle fibre[20,32]. Mercury induces oxidative stress, which causes enzymatic damage and biomolecule oxidation. Inorganic Hg is suggested to increase H_2O_2 production by impairing the efficiency of oxidative phosphorylation and electron transport at the ubiquinone-cyt b5 step[46]. MeHg also is known to be uncoupling agent stimulating state IV respiration accelerating electron transport rate in ETC; premature shedding of electrons to molecular oxygen increases and generates superoxide and hydrogen peroxide[46]. Both organic and inorganic Hg increase intracellular Ca. Calcium could activate hydrolytic enzyme such as proteases, endonucleases and phospholipases. Activation of phospholipase A2 results in increased generation of arachidonic acid which rapidly influences lipoxygenase and cyclooxygenase and results in production of superoxide. Another consequence of increased calcium is the conversion of xanthine oxidase which then catalyzes reaction with superoxidase and H_2O_2 forming[46].

Mercury could displace iron and copper from their natural binding site, therefore increases Fenton-mediated ROS formation. Gonzales et al[8] showed increase of SOD expression as response to oxidative stress in liver and skeletal muscle of danio rerio which gives dietary MeHg, but there is no change in contaminated brain sample although this organ accumulated the highest MeHg concentration. This lack of response could explain the high neurotoxicity of MeHg. Several studies have shown that mercury decreases GPx activity, whereas lipid peroxidation from the

mitochondrial membrane stimulates the production of MDA[29]. Ma et al[27] have shown that hg increases the cyt c release. Shenker et al[28] found that MeHgCl decreases GSH, increases cyt c and decreases bcl2. It is suggested that MeHgCl induces apoptosis from LPO of mitochondrial membrane.

Mitochondria and Arsenic: Arsenic (As) exists in four oxidation states: elemental arsenic (As0), arsine (AsH_3), arsenide (AsO_2^-) and arsenate (AsO_4^{3-})[29,43]. Elemental arsenic is non-toxic, whereas arsenide is ten times more toxic than arsenate. Arsenic exists in three forms: organic, inorganic and gas (arsine). Organic arsenic has low toxicity whereas inorganic and arsine gasses are toxic.

Mitochondria are arsenic target organelles. Arsenic disrupts ATP generation in several pathways. Arsenide and arsenate bind to lipoate leading to inhibition of the Krebs cycle and oxidative phosphorylation leading to ATP depletion. Arsenic inhibits the absorption of glucose in the cell, thereby inducing gluconeogenesis and oxidation of fatty acid[43]. It forms ADP-arsenate complex, which uncouples OXPHOS. Pentavalent arsenate can replace the phosphate bond in ATP with the arsenate bond, thereby rapidly hydrolysing the uncoupling OXPHOS [43]. This depleting ATP generation can induce apoptosis in high-energy dependent tissue. Several studies show that As (III) inhibits OXPHOS by decreasing complex I, II and COX activity which may reduce the ATP production to nearly 50%[14,31].

Arsenic induces stress oxidative by increasing the generation of ROS and decreases antioxidants such as SOD, GSH, CAT, GPx, GST and GR[13,14,48,57]. Arsenic induces O2 transformation into a more reactive oxygenated species such as H_2O_2 and OH-, the high amount of H_2O_2 generation through the Fenton and Haber-Weiss reaction[43]. Some reports suggest that arsine releases H_2O_2 through a spontaneous and exergonic chemical reaction[43]. The addition of CAT to the culture medium decreased the sodium arsenite toxicity which indicated a possible role of H_2O_2 in the toxicity⁴⁶⁴⁶⁴⁶⁴⁵.[46]

Other reports suggest that dimethyl arsine reacts with oxygen to form a dimethylarsenic radical and superoxide anion, addition of another oxygen onto dimetylarsenic radical results in dimethylarsenic peroxyl radical[46]. Arsenic is capable of binding to sulfhydryl (-SH), thereby decreases GSH level and decreases SOD activity to about 30% with the consequences of reducing the capabilities of mitochondria to oxidize ROS[14,15]. Prolonged administration of sodium arsenite shows decrease of SOD, CAT, GPx, GST and GR activity in the rat brain[13].

In arsenic toxication, ROS breaks the mitochondrial membrane and releases cyt c, with mitochondrial-mediated and caspase-mediated apoptosis[57]. Arsenic increases pro-apoptotic protein (cyt c and bax,) as well as decreases anti-apoptotic protein (bcl2) [14]. Several studies show an increase of LPO, increase of cyt c, caspase 3 and 9 expression in the cytosol, increase of bax/ bcl2 ratio in long term exposure sodium arsenite to the rat brains[13,14].

Mitochondria and Lead (Pb): Lead is the number one usage of the heavy metals in the world because of its ductile, malleable and corrosion-resistant properties. Lead is useful in Pb-batteries, paints, protective coatings, water pipes, glass and gasoline additives. Lead acetate is organic lead which is usually added to gasoline. Organic lead is more soluble than inorganic lead due to the high lipid solubility and the facilitated distribution within organs and tissues.[56] 95-99% of Pb binds to hemoglobin, hence spreads throughout the body[58] and the liver is the storage site and an important primary target in Pb^{2+} toxicity[36]. The hepatotoxicity of Pb could have resulted from the impairment of liver mitochondria.

Lead toxicity is associated to its ability to mimic other essential metals, most notably calcium, iron and zinc, which act as cofactors in many enzymatic reactions. Because lead does not properly function as a cofactor, it interferes with the enzymes' ability to catalyze the reaction[43,58]. Lead occupies the calcium-binding sites on numerous calcium-dependent protein and alters the protein function, thereby affects those physiological and biochemical processes of the cell that require calcium. Pb has a high affinity to sulfhydryl (-SH) groups and metal cofactor, which reduces the activities of enzymes as shown by Ma et al[36] who added Pb to liver mitochondria, Pb could inhibit mitochondrial respiratory complex III activities. Pb also contributes to the mtDNA mutation doe to a high ROS level,[47] thereby interferes the transcription of ETC complex and disrupts OXPHOS reaction.

Even though the mechanism of lead induce stress oxidative is not clear, there are several pathways associated to lead toxicity and stress oxidative. Lead inhibits delta aminolaevulinic acid synthase (δ-ALAS), delta aminolaevulinic acid dehydratase (δ-ALAD), coproporphyrinogen decarboxylase, ferro-chelatase and pyrimidine 5-nucleotidase[29]. Oxidative stress is a consequence of the inhibition of δ-ALAS and δ-ALAD enzymes. The δ-ALAS enzymes are important for the production of aminolaevulinic acid, which is bio-processed to protoporphyrin IX in the mitochondria and then chelated with iron to form heme, this process is inhibited by lead and mercury[43]. It has been reported that δ-ALAD autooxidation generates O_2^- and 4,5 diooxovaleric acid[29].

Autooxidation is a form of δ-ALAD, which acts as an electron donor and capable of transferring electrons to oxygen in oxyhemoglobin, thus forming methemoglobin, ALAD radical and H_2O_2. Interaction of O_2^- and H_2O_2 generates HO-, a powerful oxidant[59]. Lead with a dose lower than 80mg/dl stimulates the production of H_2O_2[29]. Sousa and Soares incubated yeast cells with Pb and showed that there

was an accumulated intracellular superoxide anion ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2)[60]. These kinetic studies showed that $O2^-$ production precedes the accumulation of H_2O_2, because of the leakage of electrons from the mitochondrial ETC.

Sousa and Soares[60] used an isogenic derivate ρ^0 strain, without respiratory competence (lacking mtDNA), displayed a lower intracellular ROS and higher resistance to Pb compare to the wild type strain. Thus, mitochondria are a significant source of Pb-induced ROS and the targets of its toxicity.

On other hand, Pb has a high affinity to sulfhydryl (-SH) groups and metal cofactor, which reduces the activities of antioxidant enzymes and makes oxidative stress more severe[38,39]. GSH plays a major role in protecting against free radical attack. GSH functional group -SH plays an important role in metal binding. Several studies showed that GSH is decreased in the brain, liver and eye-lens of rats exposed to lead[46]. GR is supporting the antioxidant defense by reducing glutathione disulfide (GSSG) to GSH. GR has a disulfide bond in its active site and lead interferes with disulfide bond and inhibits GR activity, thereby prevents reduction of GSSG and makes the cell more susceptible to oxidative damage.

Other antioxidant enzymes which remove peroxide and superoxide radicals such as GPx, CAT and SOD are also potential target for lead toxicity.

Lead forms a complex with selenium, meanwhile GPx required selenium for its activity, hence lead decreased GPx activity[46]. Lead indirectly inhibits heme synthesis and CAT is a heme-containing enzyme, it causes decrease of CAT activity[46,59]. Several studies in lead-exposed rats showed decreasing of RBC SOD activity[46].

Apoptosis and Pb toxicity are associated with a high ROS generation which contributes to the DNA mutation,[47] thereby inducing extrinsic apoptosis. On other hand, Pb induces mitochondrial lipid membrane peroxidation,[36] thus induces the release of cyt c and induces mitochondrial-mediated apoptosis.

Conclusion

Heavy metals induce cell toxicities by disturbance of the mitochondria. Heavy metals may alter mitochondrial ATP generation, especially towards the oxidative phosphorylation process by decreasing the protein ETC activities. Furthermore, heavy metals induce oxidative stress by increasing ROS generation and decreasing several antioxidants activity such as SOD, GPx and CAT. Heavy metals also induce MPTP opening hence causing the release of pro-apoptotic protein to the cytosol and induces apoptosis. On the other hand, heavy metals also induce apoptosis by decrease Bcl2 level, the anti-apoptotic protein. Based on the table 1, the heavy metals effect to the mitochondria depends on the kind of cell target, the chemical form of heavy metals, the dosage, the duration of exposure and if it is *in vivo* or *in vitro* experiment.

The disruption of the mitochondria as the main energy produce, causes many problems in the cell and leads to cell death. By understanding the mechanism and the role of heavy metals towards the cell function, it may give a new perspective for better treatment approach for heavy metals-induced diseases and to increase awareness of the pollution.

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