Mercury Toxicity and Antioxidants: Part I: Role of Glutathione and alpha-Lipoic Acid in the Treatment of Mercury Toxicity

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Abstract

Mercury exposure is the second-most common cause of toxic metal poisoning. Public health concern over mercury exposure, due to contamination of fish with methylmercury and the elemental mercury content of dental amalgams, has long been a topic of political and medical debate. Although the toxicology of mercury is complex, there is evidence for antioxidant protection in the prevention of neurological and renal damage caused by mercury toxicity. Alpha-lipoic acid, a coenzyme of pyruvate and alpha-ketoglutarate dehydrogenase, has been used in Germany as an antioxidant and approved treatment for diabetic polyneuropathy for 40 years. Research has attempted to identify the role of antioxidants, glutathione and alpha-lipoic acid specifically, in both mitigation of heavy metal toxicity and direct chelation of heavy metals. This review of the literature will assess the role of glutathione and alpha-lipoic acid in the treatment of mercury toxicity.
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Mercury: Sources of Exposure

According to the Agency for Toxic Substances and Disease Registry (ATSDR) of the U.S. Department of Health and Human Services, mercury is listed as the third-most frequently found (lead and arsenic are first and second), and the most toxic substance in the United States.[1] This figure originates from the U.S. Government's Priority List of Hazardous Substances. This list includes, in order of priority, substances that have been found at hazardous waste sites on the National Priorities List (Superfund sites) that "pose the most significant potential threat to human health due to their known or suspected toxicity and the frequency of exposure." Of 1,467 hazardous waste sites listed on the National Priorities List in 1998, toxic levels of mercury were identified in 714. Mercury toxicity is also considered the second-most common cause of acute heavy metal poisoning, with 3,596 cases reported in 1997 by the American Association of Poison Control Centers.[2]

Annual worldwide emissions of mercury into the atmosphere have been estimated at 2,200 metric tons.[3] One-third of these emissions are estimated to originate from natural sources (volcanic eruptions and decay of mercury-containing sediment) and two-thirds from man-made sources. Twenty-five percent of total worldwide emissions come from fossil fuel combustion. In the United States, 26 percent (64.7 tons/year) of atmospheric mercury emissions come from medical waste incineration, such as cremation.[4]

There are currently 1,782 advisories (one per body of water) issued by the U.S. Environmental Protection Agency (EPA) in 41 states in the United States restricting the consumption of any locally caught fish or shellfish due to their mercury content. Sixteen states have issued statewide or statewide-coastal advisories recommending restricting the consumption of fish caught in the state or along the coastline due to methylmercury contamination.[4] The Environmental

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Working Group, in a presentation to the Food Advisory Committee of the U.S. Food and Drug Adminstration (FDA), recently presented data warning of the consequences for fetuses of women who follow the current FDA's fish consumption advisory and eat 12 ounces of "safe" fish per week. The Environmental Working Group estimates that more than 25 percent of children *in utero* in the United States would be exposed to levels of mercury above the EPA safe reference dose (0.1 μg methylmercury/kg body weight/day) for at least 30 days during gestation and would have an increased risk for neurological damage.[5]

The ATSDR considers anyone who lives in close proximity to a former mercury mining site, recycling facility, municipal or medical incinerator, or coal-fired electric generating plant to be at risk for mercury toxicity. Anyone who routinely consumes contaminated fish, subsistence hunters who consume meat or organ tissues of marine mammals or feral wildlife, individuals with a "large number" of dental amalgams, pregnant or nursing women (and their developing fetuses and breast-fed babies), those who use consumer products containing mercury (skin-lightening creams or antiseptic facial products, mercury-containing diuretics or laxatives, and teething powders), or those living or working in buildings painted with mercury-containing latex paint are also considered at significant risk. Mercury-containing latex paint was removed from paint manufacturing in 1991 but may still be available in the reserve inventories of contractors and warehouses.[4]

Mercury is found in the environment in three basic states: elemental mercury or mercury vapor, inorganic mercury, and organic mercury (ethyl-, methyl-, alkyl-, or phenylmercury). Each form has an individual toxicological profile and metabolic fate. The most frequent source of mercury exposure is open to debate. On an individual exposure basis, the estimated intake and retention of elemental mercury vapor (from dental amalgams and atmospheric pollution) in non-occupationally exposed individuals has a much broader range (3.9-21.0 μg/day) than either inorganic (4.3 μg/day) or methylmercury (1-6 μg/day) exposure.[6]

Elemental Mercury

Elemental mercury, found in thermometers, thermostats, dental amalgams, and mercury added to latex paint, eventually enters a vaporized state. Eighty percent of inhaled elementary mercury vapor is absorbed and can cross the blood-brain barrier or reach the placenta.[2] Mercury vapor in the gastrointestinal tract is converted to mercuric sulfide and excreted in the feces.[6] Mercury vapor in the kidneys, however, the main repository for elemental mercury, is carried to all parts of the central nervous system as a lipid-soluble gas. Mercury vapor can also be oxidized to inorganic mercury by catalase and can attach to the thiol groups in most proteins – enzymes, glutathione, or almost any structural protein.[7] Elemental mercury can also be methylated by microorganisms in soil and water and potentially the human gastrointestinal tract,[8] where it can then be transformed into organic methylmercury, the form found in fish, fungicides, and pesticides. Elemental mercury and its metabolites have the toxic effect of denaturing biological proteins, inhibiting enzymes, and interrupting membrane transport and the uptake and release of neurotransmitters.[7] Chronic exposure most commonly manifests as a triad of increased excitability and irritability, tremors, and gingivitis.[2] Less commonly, chronic exposure causes central and peripheral nervous system damage, manifesting as a characteristic fine tremor of the extremities and facial muscles, emotional lability, and irritability. Rarely, significant exposure can cause acrodynia or "pink disease," involving a pink rash on the extremities, pruritis, paresthesias, and pain.[9]

Inorganic Mercury

Inorganic mercury (mercury salts) is found in cosmetic products, laxatives, teething powders, diuretics, and antiseptics.[2] Inorganic mercury can be formed from the metabolism of elemental mercury vapor or methylmercury.[7] Although inorganic mercury does not normally reach the placenta or cross the blood-brain barrier, it has been found in the neonatal brain due to the absence of a fully formed blood-brain barrier.[6] Inorganic mercury is complexed with glutathione in

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the liver and secreted in the bile as a cysteine-mercury or glutathione-mercury complex. Chronic exposure to inorganic mercury salts primarily affects the renal cortex[10] and may manifest as renal failure (dysuria, proteinuria, hematuria, oliguria, and uremia) or gastrointestinal problems (colitis, gingivitis, stomatitis, and excessive salivation). Irritability and occasionally acrodynia can occur.[2]

Organic Mercury

Considered the most toxic and most frequent form of mercury exposure, organic mercury is found in fish, poultry that has been fed fishmeal, pesticides, fungicides, insecticides, and thimerosal-containing vaccines. Thimerosal, which is 49.6-percent ethylmercury (a form of organic mercury), has been used as a preservative in vaccinations since the 1930s. It is currently mixed with DTaP, HIB, and hepatitis B vaccines or is used in the manufacturing process for vaccines, with resultant trace amounts being present in the final product. Based on existing Centers for Disease Control (CDC) recommendations for vaccinations, a typical six-month-old child, if receiving all thimerosal-containing vaccines, could potentially be injected with as much as 187.5-200 μg of methylmercury; the equivalent of more than 1.0 μg per day. This amount exceeds the reference limits for exposure to mercury set by the EPA of 0.1 μg/kg/day.[11] In the United States, at the FDA's request, all vaccines are currently being produced as thimerosal-free or thimerosal-reduced (> 95-percent reduction) vaccines. Thimerosal-preserved vaccines are still available and used in clinical practice.

Methylmercury is almost completely absorbed (95-100 percent) in the human gastrointestinal tract,[2,7] 90 percent of which is eventually eliminated through the feces. Methylmercury is present in the body as a water-soluble complex, mainly with the sulfur atom of thiol ligands,[7] and crosses the blood-brain barrier complexed with L-cysteine in a molecule resembling methionine. Methylmercury is absorbed into the placenta and stored in the fetal brain in concentrations that exceed maternal blood levels.[12] After being released from cells in a complex with reduced glutathione, methylmercury is degraded in the bile duct to an L-cysteine complex. Only 10 percent of methylmercury is eliminated through the kidneys. The rest either undergoes enterohepatic recycling or demethylation by microflora in the intestine and immune system and eventual elimination through the feces.

Most methyl mercury in animal exposure studies is degraded to, and eliminated as, inorganic mercury at the rate of one percent per day.[7] At least one study has demonstrated the capacity of two common forms of gastrointestinal yeast to convert inorganic mercury to methylmercury.[8] Demethylation by intestinal microflora is a crucial step in the elimination of methylmercury from the body, but research has not yet identified the mechanisms or the microbes responsible for this detoxification system.[7] Enterohepatic reabsorption is also a significant event in the metabolism of methylmercury; more than 70 percent is reabsorbed from the gut and returned to the liver.[7,13]

Inorganic mercury has been found as the major form of mercury in brain tissue in humans fatally exposed to methylmercury.[14] The conversion of methylmercury to inorganic mercury is thought to take place in phagocytic cells in the liver or in the astroglial cells of the brain.[7]

The majority of toxicity due to methylmercury exposure involves the central nervous system. Methylmercury can cause demyelination, autonomic dysfunction, sensory nerve conduction delay, abnormal neuronal migration, and abnormal central nervous system cell division. Chronic toxicity symptoms include paresthesia, peripheral neuropathy, cerebellar ataxia, akathisia, spasticity, memory loss, dementia, constricted vision, dysarthria, impaired hearing, smell and taste, tremors, and depression.[2,7]

Methylmercury exposure also appears to increase risk for cardiovascular disease. In a long-term prospective study, both intake of nonfatty freshwater fish and hair mercury content demonstrated a statistically significant correlation with increased risk for acute myocardial infarction.[15] Men with the highest hair mercury had a 2.9-fold increased risk for cardiovascular death. An examination of the same cohort found a significant correlation between hair mercury and increased risk

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Table 1. Mercury Species – Sources, Routes of Absorption, Distribution, and Excretion[18]

	Methylmercury	Elemental Mercury	Inorganic Mercury
Sources	Fish, poultry, pesticides	Dental amalgams, fossil fuels, old latex paint, thermometers, incinerators, occupational	Demethylation of methylmercury by intestinal microflora; biological oxidation of elemental mercury
Absorption	95-100 percent in intestinal tract; 100 percent of inhaled vapor	75-85 percent of vapor absorbed	7-15 percent of ingested dose absorbed; 2-3 percent of dermal dose absorbed in animals
Distribution	Lipophilic, distributed throughout body; readily crosses blood-brain barrier and placental barrier; accumulates in brain, kidney	Lipophilic, distributed throughout body; crosses blood-brain and placental barriers; accumulates in brain, kidney	Does not cross blood-brain or placental barrier; found in brain of neonates; accumulates in kidney
Metabolism	Cysteine complex necessary for intracellular absorption; slowly demethylated to inorganic mercury in brain by tissue macrophages, fetal liver, and free radicals	Oxidized intracellularly to inorganic mercury by catalase and hydrogen peroxide	Methylated by intestinal microflora; binds and induces metallothionein biosynthesis
Excretion	90 percent in bile,feces; 10 percent in urine	Urine, feces, sweat and saliva	Urine, bile, feces, sweat, saliva
Cause of Toxicity	Demethylation to inorganic (divalent) mercury; free radical generation; binding to thiols in enzymes and structural proteins	Oxidation to inorganic (divalent) mercury	Binding to thiols in enzymes and structural proteins

for progression of carotid atherosclerosis.[16] Prenatal exposure to methylmercury has been correlated with significant blood pressure elevations in seven-year-old children as a result of maternal fish intake.[17]

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Ethylmercury (fungicides, thimerosal in vaccines, and gamma-globulin) also causes renal and central nervous system toxicity and is deposited in the liver, kidneys, skin, brain, spleen, and plasma.[7] Ethylmercury, like methylmercury, is metabolized to the inorganic form and accounts for 50 percent of the mercury eliminated in urine. Ethylmercury may actually be converted to inorganic mercury in the tissues in greater amounts and more rapidly than methylmercury.[7] As with methylmercury, the feces are the main natural route of elimination. Table 1 summarizes the forms of mercury and their pharmacokinetics.

Mechanisms of Mercury Toxicity

Mercury can cause biochemical damage to tissues and genes through diverse mechanisms, such as interrupting intracellular calcium homeostasis, disrupting membrane potential, altering protein synthesis, and interrupting excitatory amino acid pathways in the central nervous system.[19] Mitochondrial damage, lipid peroxidation, microtubule destruction,[20] and the neurotoxic accumulation of serotonin, aspartate, and glutamate are all mechanisms of methylmercury neurotoxicity.[19]

Over time, both methylmercury and elemental mercury vapor in the brain are transformed to inorganic mercury, and become firmly bound to sulfhydryl-containing macromolecules.[21] Both methylmercury and inorganic mercury bind to various molecular weight thiol-containing proteins (glutathione, cysteine, albumin, etc.). The binding and dissociation of these mercury-thiol complexes are believed to control the movement of mercury and its toxic effects in the body.[7]

Mitochondrial damage from oxidative stress may be the earliest sign of neurotoxicity with methylmercury. A study in neural tissue indicates the electron transport chain appears to be the site where free radicals are generated, leading to oxidative damage induced by methylmercury.[19]

Mercury-Thiol Binding

Because the stability constants (energy necessary to form and break bonds) for mercury and thiol complexes (glutathione, albumin, cysteine, etc.) are so high, mercury will bind to any free thiol available and the thiol in the highest concentration will be the most frequently-bound.[22] The reaction rate is almost instantaneous.[7] Although the mercury-sulfhydryl bond is stable, it is labile in the presence of other free sulfhydryl groups; therefore, methylmercury will be redistributed to other competing sulfhydryl-containing ligands.[23] This is the basis for chelation of heavy metals with sulfhydryl compounds like DMPS and DMSA – providing free sulfhydryl groups in high concentrations to encourage the metal to move from one sulfhydryl-containing ligand to another.

The endogenous thiol-containing molecules – glutathione, cysteine, homocysteine, metallothionein, and albumin – all contain reduced sulfur atoms that bind to mercuric ions and determine the biological fate of mercury compounds in the body.[24] The complex of methylmercury and cysteine may act as a "molecular mimic" for the amino acid methionine and gain entry into the central nervous system via the same mechanism methionine uses to cross the blood-brain barrier.[25] Endogenous thiols transport mercury compounds and act to protect them from binding to other proteins, preventing functional damage in that tissue. In general, the higher the cysteine or thiol concentration in a cell medium, the lower the concentration of intracellular divalent mercury. In other words, higher concentrations of thiols appear to protect against accumulation of mercury, both *in vivo* and *in vitro*.[22]

Glutathione in Heavy Metal Binding

Glutathione is the most common low-molecular weight sulfhydryl-containing compound in mammalian cells, present in millimolar amounts in most cells.[26] As a result of the binding of mercury to glutathione and the subsequent elimination of intracellular glutathione, levels of reduced glutathione are lowered in several specific types of cells on exposure to all forms of mercury. Glial cells,[27] human erythrocytes,[28] and mammalian renal tissue[24] have all been found to have significantly lowered levels

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of reduced glutathione, a major source of oxidant protection. Mercury, as well as cadmium, generates highly toxic hydroxyl radicals from the breakdown of hydrogen peroxide, which further deplete glutathione stores.[27] There is evidence that glutathione depletion can lead to neurological damage; low levels of glutathione have been found in Parkinson's disease and cerebral ischemia-reperfusion injury.[29]

Glutathione, as both a carrier of mercury and an antioxidant, has three specific roles in protecting the body from mercury toxicity. First, glutathione, specifically binding with methylmercury, forms a complex that prevents mercury from binding to cellular proteins and causing damage to both enzymes and tissue.[30] Glutathione-mercury complexes also reduce intracellular damage by preventing mercury from entering tissue cells and becoming an intracellular toxin.

Second, glutathione-mercury complexes have been found in the liver, kidney, and brain, and appear to be the primary form in which mercury is transported and eliminated from the body.[24] The transport mechanism is unclear, but complexes of glutathione and mercury are the predominant form of mercury in both the bile and the urine.[31] Glutathione and cysteine, acting as carriers of mercury, actually appear to control the rate of mercury efflux into bile; the rate of mercury secretion in bile appears to be independent of actual bile flow. When bile flow rate is increased or decreased, the content of mercury in the bile changes inversely so net mercury efflux from the liver remains unchanged.[32] However, increasing bile levels of both glutathione and cysteine increases the biliary secretion of methylmercury in rats.[13] Other studies have confirmed this data in animal models.[33-35] Conversely, glutathione depletion inhibits biliary secretion of methylmercury in animal models and blocking glutathione production appears to shut down biliary release of mercury.[35]

Cells of the blood-brain barrier (brain capillary endothelial cells) release mercury in a glutathione complex. Inhibiting glutathione production in these cells inhibits their ability to release mercury.[23] Mercury accumulates in the central nervous system primarily in astrocytes, the cells that provide the first line of defense for the central nervous system against toxic compounds.[36] Astrocytes are the first cells in brain tissue to encounter metals crossing the blood-brain barrier. They also contain high levels of metallothionein and glutathione, both carriers for heavy metals. It is hypothesized that astrocytes are the main depot of mercury in the brain.[37] In studies with astrocytes, the addition of glutathione, glutathione stimulators, or glutathione precursors significantly enhances the release of mercury from these cells in a complex with glutathione. Fujiyama et al[38] also suggest that conjugation with glutathione is the major pathway for mercury efflux from astrocytes. Glutathione also increases mercury elimination from renal tissue. Studies in mammalian renal cells reveal glutathione is 50 percent as effective as the chelating agent DMSA (2,3-dimercaptosuccinic acid) in preventing inorganic mercury accumulation in renal cells.[39]

Third, glutathione increases the antioxidant capacity of the cell, providing a defense against hydrogen peroxide, singlet oxygen, hydroxyl radicals, and lipid peroxides produced by mercury.[30] The addition of glutathione to cell cultures exposed to methylmercury also prevented the reduction of cellular levels of glutathione peroxidase, a crucial antioxidant enzyme necessary for protection against the damaging effects of lipid peroxidation.[30]

As an antioxidant, glutathione appears to protect against renal damage resulting from inorganic mercury toxicity. The co-incubation of rat renal cells with glutathione and inorganic mercury was significantly more protective of renal cell injury when compared to inorganic mercury exposure alone.[40] Antioxidant levels – specifically glutathione, vitamin E, and ascorbic acid – are depleted in renal tissue exposed to mercuric chloride (inorganic mercury), and the addition of glutathione increased levels of both vitamin E and ascorbic acid in renal cells exposed to mercuric chloride.[24]

Mammalian cell lines resistant to mercury toxicity have been cloned.[41] They do not readily accumulate mercury and are resistant to the toxic

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effects of methylmercury or inorganic mercury. An outstanding characteristic of this cell line is that glutathione levels are five times greater in these cells than the parent cells from which they originated. The authors of this study conclude that the mechanisms of resistance were primarily due to glutathione's ability to facilitate mercury efflux from cells and the protective binding of mercury by glutathione to prevent cellular damage.

The Role of alpha-Lipoic Acid

In 1966, German physicians began using alpha-lipoic acid (ALA) therapeutically in patients with diabetic polyneuropathy and liver cirrhosis because of their observation that these patients had lower levels of circulating lipoic acid.[42] The application was subsequently extended to heavy metal intoxication and toxic mushroom poisoning.

According to Jones and Cherian,[43] an ideal heavy metal chelator should be able to enter the cell easily, chelate the heavy metal from its complex with metallothionein or other proteins, and increase the excretion of the metal without its redistribution to other organs or tissues. Although no human clinical trial has investigated the use of ALA as a chelating agent in mercury toxicity, there is evidence ALA satisfies at least two of the above criteria; i.e., absorption into the intracellular environment and complexing metals previously bound to other sulfhydryl proteins.

ALA produced endogenously is bound to proteins, but can also be found unbound in the circulation, after exogenous lipoic acid supplementation.[41] In this form it is chemically able to trap circulating heavy metals, thus preventing cellular damage caused by metal toxicity.[41] Lipoic acid is lipophilic and is able to penetrate cell membranes and reach high intracellular concentrations within 30 seconds of its administration.[44]

The fact that free ALA crosses the blood-brain barrier is significant because the brain readily accumulates lead and mercury, where these metals are stored intracellularly in glial tissue.[36,45] Oral doses of 10 mg/kg ALA in rats have reached peak levels in the cerebral cortex, spinal cord, and peripheral nerves within 30 minutes of administration, and studies of chronic daily dosing conclude ALA reaches all areas of the CNS and peripheral nervous system.[46] ALA has been shown to decrease lipid peroxidation in brain and sciatic nerve tissue[47] and when given orally to rats, decreased lipid peroxidation in brain tissue by 50 percent.[46] In diabetic neuropathy, free lipoic acid may prevent glucose-related oxidative damage by entering nerve tissue where it acts as both an antioxidant and heavy metal-binding agent.[28]

ALA has been administered to humans in doses up to 1,200 mg intravenously without toxicity, and in oral daily doses of as much as 600 mg three times daily. The only side effects reported are infrequent nausea and vomiting. No side effects have been reported in oral administration of up to 1,800 mg daily.[41,48] Doses of 500-1,000 mg have been well tolerated in placebo-controlled studies.[49] Extrapolation of pharmacokinetic and toxicity data demonstrate safe human dosages would not be exceeded with oral doses of several grams per day.[41]

ALA has been shown to increase both intra- and extracellular levels of glutathione in T-cell cultures, human erythrocytes, glial cells, and peripheral blood lymphocytes.[50] In rats, oral dosing of 150 mg/kg/day for eight weeks significantly increased glutathione levels in the blood and liver.[51] ALA has been shown to increase intracellular glutathione by 30-70 percent in murine neuroblastoma and melanoma cell lines, and in the lung, liver, and kidney cells of mice that had received intraperitoneal injections of 4, 8, or 16 mg/kg ALA for 11 days.[52,53] Levels of intracellular glutathione have been shown to increase by 16 percent in T-cell cultures at concentrations of 10-100 μM (concentrations achievable with oral and intravenous supplementation of ALA).[50] A single oral dose of 600 mg ALA was able to produce a serum concentration of 13.8 ± 7.2 μM and levels of 100-200 μM have been reported after 600 mg intravenous administration.[54]

Increases in glutathione levels seen with ALA administration are not only from the reduction of oxidized glutathione (one of the functions of ALA) but also from the synthesis of glutathione.[46] ALA is reduced to dihydrolipoic acid

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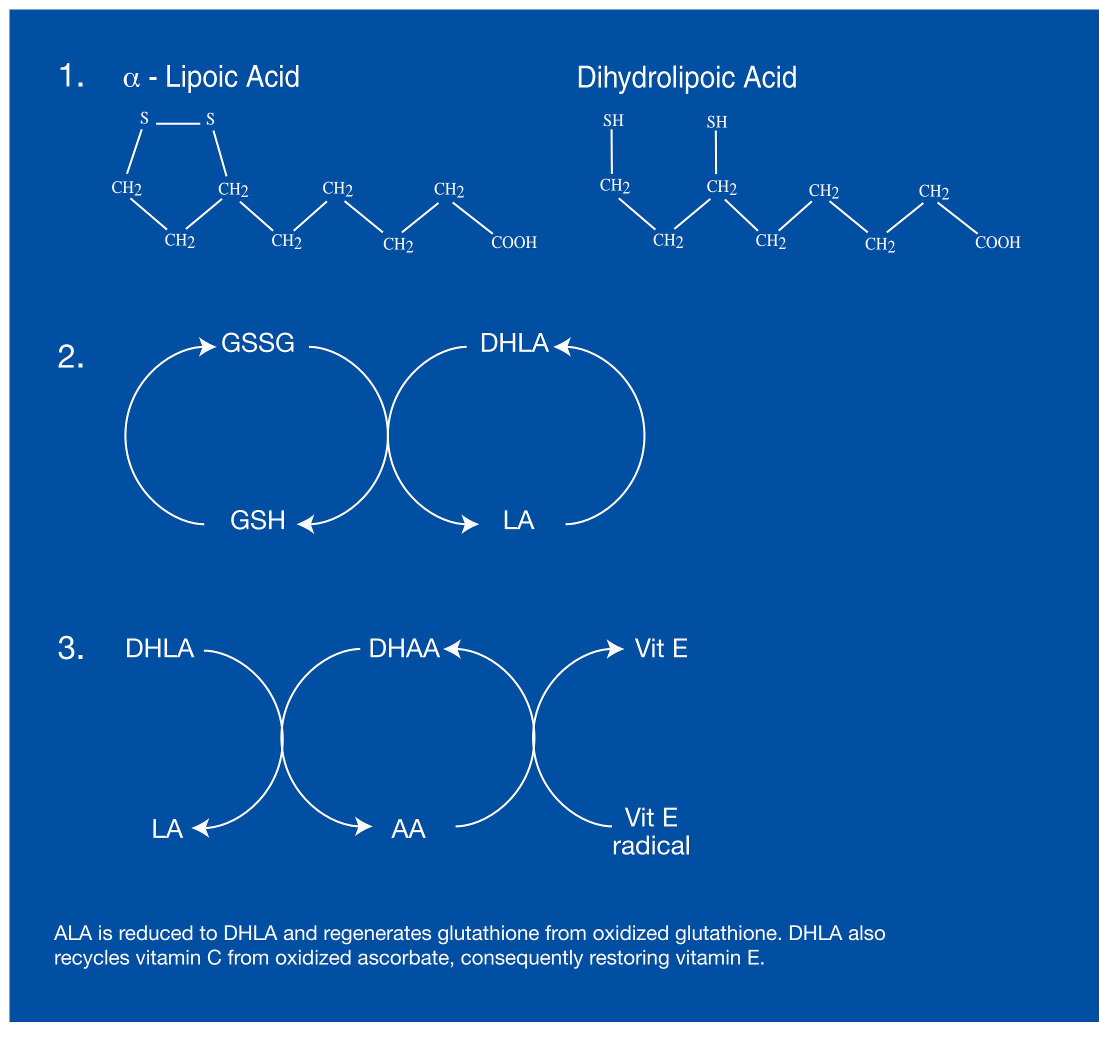
Figure 1. Antioxidant Recycling



ALA is reduced to DHLA and regenerates glutathione from oxidized glutathione. DHLA also recycles vitamin C from oxidized ascorbate, consequently restoring vitamin E.

(DHLA), itself a potent antioxidant. DHLA is able to regenerate oxidized ascorbate, glutathione, coenzyme Q, and vitamin E,[28] and is responsible for the ability of ALA to increase intracellular glutathione levels (Figure 1).[55]

ALA, through its reduction to DHLA and oxidation back to ALA, has the ability to continuously provide cysteine, the rate-limiting amino acid for glutathione production. ALA is rapidly reduced to DHLA and released in the extracellular environment where it reduces extracellular cystine to cysteine and increases the uptake of cysteine into the cell,[50] increasing glutathione production. ALA does this through enzyme-catalyzed reactions using NADH or NADPH, the metabolic power resulting from glucose metabolism (Figure 2).[51]

ALA and Binding of Copper, Iron, Platinum, and Lead

ALA and DHLA have been shown to form complexes with manganese ($Mn2^{+}$), zinc ($Zn2^{+}$), cadmium ($Cd2^{+}$), lead ($Pb2^{+}$), cobalt ($Co2^{+}$), nickel ($Ni2^{+}$), and iron ($Fe2^{+}$) ions.[55] In many cases, ALA-mediated heavy-metal binding prevents free-radical caused tissue damage or enzyme inactivation.[56]

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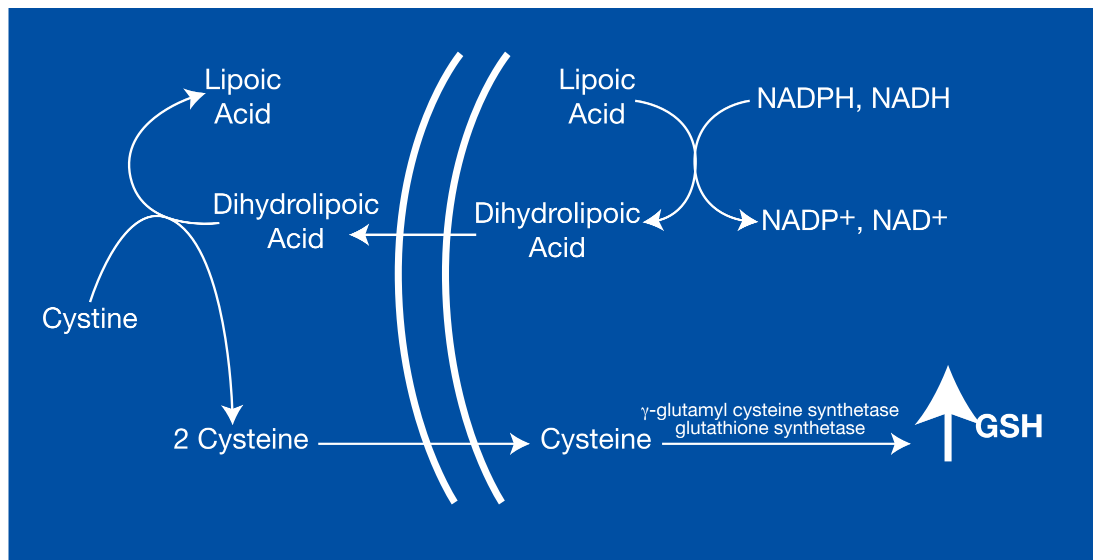
Figure 2. Reduction of ALA to DHLA and Cystine to Cysteine



In the case of iron and copper, complexing with ALA can protect cells from damage caused by iron- or copper-induced lipid peroxidation.[41] ALA has been shown to bind copper in human lipoproteins[57] and, as a result, to inhibit copper-induced peroxidation of low density lipoproteins. ALA has been used to treat Wilson's disease, effectively increasing renal copper excretion and normalizing liver function.[58]

ALA is also able to form complexes with ferritin-bound iron both *in vitro* and *in vivo*.[59] ALA has the ability to displace protein or vitamin C bound to iron and bind to $Fe2^{+}$. DHLA can facilitate the release of iron from the ferritin molecule and bind iron.[41]

The brain, particularly the substantia nigra and the globus pallidus, contains high levels of iron.[46] The high iron content and an increased level of unsaturated fatty acids lead to increased levels of tissue peroxidation.[46] ALA has been found to suppress the free radicals initiated by reactions with iron in the substantia nigra and other parts of the CNS.[46]

ALA has also been shown to protect against cisplatin-induced renal damage in rats by binding to platinum that is responsible for renal toxicity.[60] At dosages of 25-100 mg/kg (equivalent to 7 grams per 70 kg human adult), ALA restored normal levels of antioxidant enzyme activity, increased reduced glutathione levels, and significantly decreased renal tissue platinum content. The dose of cisplatin used in the study (16 mg/kg) is similar to clinical use in cancer treatment. Although the potential toxicity of this high dose of ALA is unknown, it is much higher than the 300-1800 mg typically used clinically.[46,48,49]

An intraperitoneal injection of 25 mg/kg ALA given to rats for seven days was able to significantly alter the oxidative stress induced by lead toxicity.[61] ALA administration increased glutathione levels 207 percent in the lead-exposed rats and decreased malondialdehyde levels in the brain, kidneys, and red blood cells, three of the four main targets of lead toxicity.[61] Further studies in cell lines of the fourth target, the reproductive system, found ALA had a protective effect in hamster ovarian cells, decreasing oxidative stress that causes cellular damage and death as a result of lipid peroxidation.[61] Because lead exposure was high (2,000 ppm injected daily into rats for five weeks) and the length of time ALA was administered was short (seven days), there may not have been

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enough time to see decreases in levels of lead in the brain or kidneys, if that effect were to take place. There were significant improvements in cell viability in ovarian cells exposed to lead that did not result from direct ALA-iron binding, suggesting ALA has a protective effect in lead toxicity aside from its ability to bind and excrete lead.[61]

ALA and Cadmium, Arsenic, and Mercury

Cadmium, arsenic, and mercury toxicity all involve similar pathways of cellular damage; i.e., mitochondrial damage, inhibition of mitochondrial enzymes, suppression of protein synthesis, and production of free radicals.[62] All three have a strong affinity for sulfhydryl-containing ligands (glutathione, alpha-lipoic acid, etc.), and each result in depressed levels of reduced glutathione.[63] The efficacy of ALA as an antioxidant and heavy metal-complexing agent in cadmium, arsenic, and mercury toxicity has been studied in animals – with results that may be applicable to heavy metal toxicity in humans.

ALA, at concentrations of 5 mM, was able to protect rat hepatocytes from cadmium toxicity (200 μM) by preventing decreases in total glutathione and increases in lipid peroxidation.[63] Another cadmium study investigated 1.5-6.0 mM concentrations of ALA or 17-89 μM DHLA in rat hepatocytes exposed to cadmium.[64] Both protocols decreased cadmium uptake by hepatocytes and normalized hepatocyte glutathione levels, leading to increased cell viability and survival despite the cadmium toxicity. ALA has also been shown (at a 30 mg/kg injected dose) to completely prevent damage that occurs from cadmium-induced lipid peroxidation in rat brain, heart, and testes.[65] In addition, ALA completely restored glutathione levels in the rat brain that had declined 63 percent with cadmium exposure.

A frequently quoted article referring to ALA as a heavy metal-complexing agent is the study by Grunert.[66] Published in 1960, the investigation used a dog and rat model in which simultaneous injection of sodium arsenate and ALA in both animals protected them from fatal arsenic toxicity. It has been shown that in acute arsenic intoxication, lipoic acid can form a complex with arsenic that renders the arsenic nontoxic.[41] Studies dosing mice with arsenic have shown ALA prevents intestinal uptake of arsenic and reduces the toxic effect of arsenic on enzyme inhibition.[49]

ALA has been shown to affect the release of glutathione into bile secretions. In animal studies, increasing amounts of glutathione in bile has been shown to dramatically increase the release of inorganic mercury. ALA given intravenously to rats at doses of 37.5-300 μM/kg was shown to increase inorganic mercury release in bile by 1,200-4,000 percent immediately after mercury exposure.[67] Levels of released inorganic mercury remained at a 300-700 percent elevation, even three hours after dosing with ALA. If mercury was injected 24 hours prior to the administration of ALA, the increase in release of inorganic mercury was substantially less, but was still elevated 140-330 percent. A lower dose of ALA (37.5 μM/kg) was more effective than higher doses at increasing the biliary elimination of methylmercury.

There was disconcerting evidence from this study, however, that ALA may also alter the tissue distribution of mercury and other heavy metals.

Although levels of inorganic mercury and methylmercury in the kidney dropped significantly, levels of inorganic mercury also increased significantly in the brain, lung, heart, and liver tissue. Methylmercury levels had also increased in the brain, intestine and muscle of the rats given ALA. The same phenomenon occurred in rats exposed to cadmium and given the same doses of ALA. Levels of cadmium in the liver dropped (where cadmium is most frequently stored) but increased in the kidney and muscle. The same was true in rats given copper and ALA; all tissues examined had increased levels of copper, except for the liver (where copper usually accumulates) where levels had dropped.[67] In all cases the pattern was the same; the tissues that concentrated the metal (blood, spleen, and kidneys in the case of methylmercury) had reduced concentrations, while other tissues appeared to have a greater concentration.

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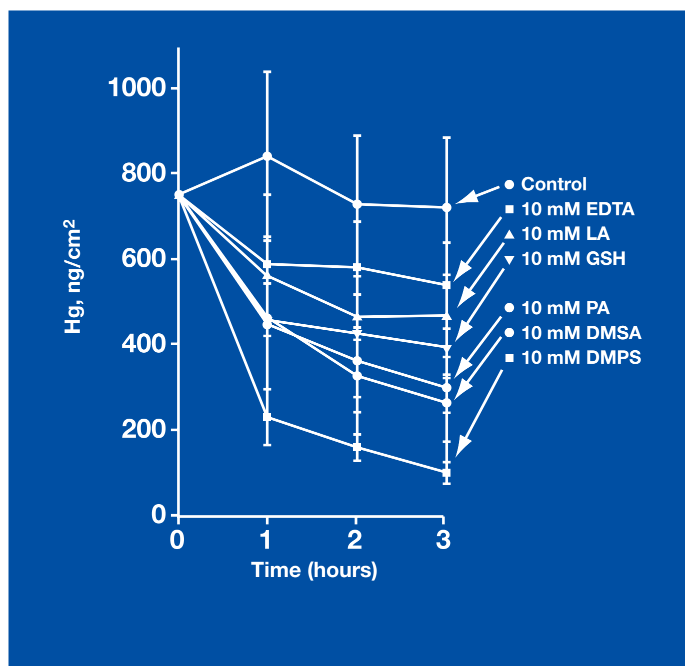
Figure 3. Ability of Chelating Agents to Lower Mercury Content of Renal Tissue in vitro *from Rabbits Injected with Mercuric Chloride*



Grunert subjected mice to lethal doses of mercuric chloride accompanied with ALA.[66] He found the ALA-to-mercury ratio was crucial in determining the outcome. A ratio of 6-8 moles ALA per mole mercuric chloride was necessary to allow the mice to survive mercury poisoning. A lower level of ALA actually increased the mercury toxicity (a molar ratio of 2 moles ALA to 1 mole mercuric chloride or lower) above control levels. The level of mercuric chloride used in this experiment, 20 mg/kg, is high and would only be seen in acute mercury poisoning.

In another study of mercury intoxication, an injection of 10 mg/kg/day ALA in rats given an injection of 1 mg/kg/day mercuric chloride prevented damage to nerve tissue caused by lipid peroxidation.[68] ALA significantly reduced lipid peroxidation in the mercury-exposed rats while elevating levels of the antioxidants glutathione, ascorbate, and tocopherol. The mechanism of protection was hypothesized to be the scavenging of peroxyl radicals formed in the brain and nervous system, although the authors believed direct complexing of inorganic mercury by ALA was also a possibility.

ALA versus Dithiol-based Chelating Agents (DMPS, DMSA)

The ability of ALA to bind inorganic mercury from rabbit renal tissue was compared to glutathione and the chelators 2,3-dimercaptopropane-1-sulfonate (DMPS), meso-2,3-dimercapto- succinic acid (DMSA), penicillamine, and ethylenediaminetetra acetic acid (EDTA) (Figure 3).[69]

DMPS was the most efficient chelator, removing 86 percent of the mercury in three hours, with DMSA being the next-most efficient, removing 65 percent of the mercury. In the same time period, penicillamine removed 60 percent, glutathione removed 50 percent, ALA removed 35 percent, and EDTA removed 20 percent. Only the levels reached by DMSA and DMPS, however, were statistically significantly different from baseline ($p<0.05$). Therefore, the effect of ALA and glutathione may show only a trend or an apparent effect and are not comparable to DMPS and DMSA. Although the actual effect of a chelator or heavy metal-complexing agent cannot be determined in a three-hour time period, and acute doses of 10 mg/kg of inorganic mercury would be considered highly toxic in an adult human, there is evidence from this study that ALA is a less efficient binder of inorganic mercury than the recognized chelating agents, DMSA and DMPS. All of the substances were used at a concentration of 10 mM, a level difficult to reach with ALA oral supplementation.

In another comparison study, ALA (25 mg/kg/day) resulted in an insignificant decrease in blood and tissue lead in rats with lead toxicity when compared to the dithiol-based chelating agent, DMSA (dosed at 90 mg/kg/day) (Table 2).[61]

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Table 2. Blood Lead Levels from Fischer 344 Rats

	Control	Pb only	Pb + LA	Pb + DMSA
Blood lead levels (mcg/dL)	0.2 ± 0.5	36.4 ± 4.4*	28.7 ± 4.1	2.0 ± 1.0**

All values represent mean ± SD for 5-10 samples
*p < 0.001, compared to the corresponding value of control group
** p < 0.005, compared to the corresponding value of lead group

Both DMSA and DMPS have been shown to be clinically effective heavy metal chelators in human studies of mercury toxicity,[70-75] particularly since they both chelate inorganic and organic mercury.[71] DMSA acts only as an extracellular chelator, whereas DMPS enters hepatocytes[73] and renal cells,[76] although it is still considered primarily an extracellular chelator.[73] DMSA is less toxic because of its inability to enter cells or bile,[73] with an LD_{50} of 13.73 mM/kg, approximately twice the LD_{50} of DMPS, which is 6.53 mM/kg.[73] While DMSA has been found to be more effective than DMPS at removing mercury from the brain,[77] DMPS appears to be more effective at removing mercury from the kidney.[78]

Conclusion

Many unanswered questions remain regarding ALA and heavy metal detoxification, especially pertaining to mercury. The amount of ALA supplemented versus the amount of toxic metal stored in the tissues is important, and has been clearly detailed in animal trials. A molar ratio of 6-8:1 (ALA:mercury) is necessary for protection and viability in mercury studies; a ratio of 2:3 has been seen in arsenic studies.[66] The ability of ALA to assist or prevent movement of heavy metals from the liver appears to be element-specific. In a previously mentioned study, the biliary release of methylmercury, cadmium, zinc, and copper was inhibited by ALA.[69]

The evidence that ALA may mobilize heavy metals to other tissues from tissues where the metals are most concentrated, specifically the brain, is troublesome. An explanation for this finding may lie in the complexing of heavy metals with glutathione and lipoic acid. Inorganic mercury forms stable complexes with ALA or DHLA and could be excreted with DHLA independent of available glutathione.[67] As Gregus et al[67] hypothesize, injected lipoic acid could complex with glutathione as it passes through the liver, preventing glutathione from carrying other heavy metals such as cadmium, or transition metals such as zinc and copper, into bile. Speculation aside, there is clear evidence ALA and its reduced form DHLA have the ability to act as both intra- and extracellular heavy metal-complexing agents, with little known toxicity and patterns of heavy metal mobilization and transport not yet understood in humans. In the absence of data from human trials, however, it can only be suggested that ALA be used as an adjunct to chelation with the standard dithiols, DMPS and DMSA.

Mercury toxicity is a significant clinical entity, as it is ubiquitous in the environment and poses serious risk to human health. The pathology of mercury toxicity in humans is diverse and encompasses direct damage to tissues and enzyme function as well as indirect damage as a result of oxidant stress.

Glutathione has been shown to be a significant factor in heavy metal mobilization and excretion, specifically with application to mercury, cadmium, and arsenic. Glutathione depletion and glutathione supplementation have specific effects on mercury toxicity, both by altering antioxidant status in the body and by directly affecting excretion of mercury and other heavy metals in the bile.

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Lipoic acid has been shown, by its increasing of cellular glutathione levels, to support the mobilization and excretion of mercury, and to decrease cellular damage and neurotoxicity. The reduced form of ALA, DHLA, appears to have direct heavy metal-binding effects. When compared to pharmaceutical dithiol-chelating agents, ALA appears to be able to bind and mobilize heavy metals from tissue, although with much weaker an effect.

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