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Review

The role of thiols, dithiols, nutritional factors and interacting ligands in the toxicology of mercury

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Abstract

Mercury has been known as a toxic substance for centuries. Whilst the clinical features of acute mercury poisoning have been well described, chronic low dose exposure to mercury remains poorly characterised and its potential role in various chronic disease states remains controversial. Low molecular weight thiols, i.e. sulfhydryl containing molecules such as cysteine, are emerging as important factors in the transport and distribution of mercury throughout the body due to the phenomenon of “Molecular Mimicry” and its role in the molecular transport of mercury. Chelation agents such as the dithiols sodium 2,3-dimercaptopropanesulfate (DMPS) and *meso*-2,3-dimercaptosuccinic acid (DMSA) are the treatments of choice for mercury toxicity. Alpha-lipoic acid (ALA), a disulfide, and its metabolite dihydrolipoic acid (DHLA), a dithiol, have also been shown to have chelation properties when used in an appropriate manner. Whilst *N*-acetyl-cysteine (NAC) and glutathione (GSH) have been recommended in the treatment of mercury toxicity in the past, an examination of available evidence suggests these agents may in fact be counterproductive. Zinc and selenium have also been shown to exert protective effects against mercury toxicity, most likely mediated by induction of the metal binding proteins metallothionein and selenoprotein-P. Evidence suggests however that the co-administration of selenium and dithiol chelation agents during treatment may also be counter-productive. Finally, the issue of diagnostic testing for chronic, historical or low dose mercury poisoning is considered including an analysis of the influence of ligand interactions and nutritional factors upon the accuracy of “chelation challenge” tests.

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Keywords: Mercury; Thiols; DMPS; DMSA; Lipoic acid; Fibre

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1. Introduction

Mercury toxicity is a subject of increasing interest and controversy in modern medicine. Though mercury has been known as a toxic substance for hundreds of years, much remains to be elucidated on the mechanisms by which mercury interacts and interferes with the body's biochemical processes. Whilst debate over the use of mercury in dental amalgams has continued for decades, more recent controversy has arisen over the use of the mercury-containing preservative thimerosal in vaccines and also over exposure to organic mercury through fish consumption. There has also been speculation as to whether exposure to heavy metals such as mercury might form part of the aetiology of various neurodegenerative diseases such as amyotrophic lateral sclerosis, Alzheimer's disease, multiple sclerosis and Parkinson's disease (Clarkson, 2002; Mutter et al., 2004). Additionally, there has been considerable interest in the possible role of the ethylmercury-containing thimerosal, in the aetiology of neurodevelopmental disorders such as autism (Geier and Geier, 2006, Mutter et al., 2004; Parker et al., 2004).

Each of the issues raised above relate to chronic low-level mercury toxicity, about which there is a paucity of data—a maximum safe exposure level has yet to be determined (Berlin, 2003; Risher and Amler, 2005). Whilst the clinical toxicology of the various forms of acute and chronic mercury toxicity has been well described in recent reviews (Clarkson, 2002; Clarkson et al., 2003), and while the advances in understanding of the molecular biology of mercury toxicology has been the focus of other reviews (Bridges and Zalups, 2005; Zalups, 2000),

there is currently a lack of congruency between the two sub-specialties.

The aim of this review is to seek such congruency by considering the clinical, diagnostic and therapeutic implications of this improved understanding of the molecular biology of mercury toxicity. This review specifically focuses on the influence of thiols, dithiols and interacting ligands such as zinc and selenium containing proteins on the toxicity of mercury at the molecular level (see Table 1 for summary). It also assesses the implications of these molecules on the clinical testing of mercury toxicity, considered within the context of chronic long-term sub-clinical exposure to the different forms of mercury with the likely selective retention by the brain of inorganic mercury over time.

2. Forms of mercury

2.1. Elemental mercury/metallic mercury/Hg⁰

Exposure to mercury comes from several sources and mercury comes in several different forms. Elemental mercury (Hg⁰) is not well absorbed upon ingestion but is well absorbed upon inhalation. Elemental mercury is used in thermometers, dental amalgams and is used in several other domestic and industrial applications. When left standing, metallic mercury volatilises at room temperature to form a vapour that is well absorbed by the lungs. Once absorbed this form of mercury is lipid soluble, can cross the blood brain barrier and placenta and can be oxidised by catalase and hydrogen peroxide into inorganic mercury (Hg²⁺), which is retained by the brain for years (Braunwald et al., 2001; Hargreaves et al., 1988; Opitz et al., 1996; Takeuchi et al., 1989; Vahter et

Table 1
Summary table of agents used in the treatment of mercury toxicity

Molecule	Type	Role in the treatment of Hg toxicity	Other biological functions
Zn	Mineral	Induces production of the metal-binding protein, metallothionein, which in turn is thought to be neuroprotective on exposure to Hg vapour	Involved in synthesis and stabilization of proteins, DNA and RNA. Structural role in ribosomes and membranes. Role in binding of steroid hormones and transcription factors to DNA, gene transcription. Essential for normal spermatogenesis, fetal growth and embryonic development. Competitive inhibitor of copper absorption*
Se	Mineral	Shown to affect the distribution and reduce the toxicity of Hg in animals. However, there is evidence of negative interactions with the dithiol chelation agents DMPS and DMSA in animals with Hg toxicity	In the form of selenocysteine, component of the enzyme glutathione peroxidase and deiodinase enzymes. Selenium has a narrow therapeutic index with toxicity beginning at doses of $\geq 400 \mu\text{g/day}$ *
NAC	Endogenous thiol	Known to boost GSH levels. Used by some physicians to treat Hg toxicity as GSH has been shown to increase biliary excretion of methyl-Hg. However, experiments have also shown that both NAC and GSH are involved in the biochemical uptake mechanism of Hg by the brain and kidneys	Is an antioxidant. IV NAC used as an antidote to acetaminophen overdose*. In spray form is used as a mucolytic—acts by splitting disulfide bonds in mucoproteins. Is taken orally to protect against contrast induced nephropathy†
GSH	Endogenous thiol	Involved in biliary excretion of methyl-Hg. Thought that intracellular GSH plays a role in protecting cells. Conversely, has also been shown to be involved in renal uptake of both inorganic Hg and methyl Hg	Antioxidant that acts as intracellular free radical scavenger. In G6PD deficiency (an enzyme deficiency), a failure to regenerate glutathione in times of increased oxidative stress leads to lysis of red blood cells‡
ALA	Endogenous disulfide	Metabolised intracellularly to DHLA (a dithiol). Shown to have protective effects against mercury toxicity in several mammal species. Dose size and frequency appear to be important with inappropriate dosing seemingly increasing toxicity. Can access all tissues of the body including the brain	Coenzyme for the enzyme complexes: pyruvate dehydrogenase, α -ketoglutarate dehydrogenase and branched-chain α -keto acid dehydrogenase‡. Increases intracellular glutathione levels. Regenerates vitamins C and E
DMPS	Synthetic dithiol	Binds tightly to inorganic Hg molecules. Due to its low molecular weight is readily filtered by the kidneys and is excreted via the urine. Does not chelate mercury in the brain	Chelator of other heavy metals including arsenic, lead and cadmium. Known to chelate the essential minerals copper, chromium and zinc. Has been used in the treatment of Wilson's disease§
DMSA	Synthetic dithiol	Binds tightly to inorganic Hg molecules. Due to its low molecular weight is readily filtered by the kidneys and is excreted via the urine. Does not chelate mercury in the brain	Chelator of other heavy metals including arsenic, lead and cadmium. Known to chelate the essential minerals copper and zinc. Labelled with a radionuclide is used in nuclear medicine§

Key: Hg = mercury; Zn = zinc; Se = selenium; NAC = *N*-acetylcysteine; GSH = glutathione; ALA = alpha-lipoic acid; DHLA = dihydrolipoic acid; DMPS = Na 2,3-dimercaptopropanesulfate; DMSA = *meso*-2,3-dimercaptosuccinic acid; G6PD = glucose 6-phosphate dehydrogenase.

* Braunwald et al. (2001).

† Tepel et al. (2000).

‡ Champe et al. (2005).

§ Sweetman (2002).

al., 1994). It is worth noting that dental amalgam emits mercury vapour, which is inhaled and absorbed into the bloodstream (Braunwald et al., 2001; Clarkson et al., 2003).

2.2. Inorganic mercury/Hg²⁺

Inorganic mercury (Hg²⁺) is found in several cosmetic and household products (Ozuah, 2000) as well as

being used industrially. It is well absorbed upon ingestion and through the skin. It can be formed as a metabolite of elemental mercury vapour (on entering the cell), methylmercury and ethylmercury (more rapidly than methylmercury) (Clarkson, 2002). Relatively little mercury in inorganic form crosses the blood brain barrier; most is excreted in the urine or faeces, or is retained by the kidneys. However, inorganic mercury can be formed in the brain as a metabolite of other forms of mercury

where it can remain for years (Takeuchi et al., 1989; Vahter et al., 1994).

2.3. Organic mercury

Organic mercury exposure in humans commonly comes in two main forms: methylmercury (CH_3Hg^+) from the consumption of fish; ethylmercury ($\text{C}_2\text{H}_5\text{Hg}^+$) which is a component of the preservative thimerosal used in vaccines. Organic mercury can undergo pulmonary absorption and is well absorbed upon ingestion. Only small amounts are absorbed through the skin. The safety of thimerosal is currently an issue of debate. Organic mercury readily crosses the blood brain barrier and the placenta, appears in breast milk, and concentrates in the kidneys and central nervous system (Braunwald et al., 2001).

Dimethylmercury, $(\text{CH}_3)_2\text{Hg}$, is a form of organic mercury encountered only in the laboratory. It is of note as a “supertoxic” compound that is strongly absorbed through the skin, (NB: latex gloves do not offer protection) and readily forms a vapour. Exposure to an amount equivalent to a few drops is lethal, with cerebellar degeneration as its prominent feature (Braunwald et al., 2001; Nierenberg et al., 1998). In 1997 it resulted in the death of a university chemistry professor and its use in the laboratory is strongly advised against if alternative mercury compounds are available (Nierenberg et al., 1998).

3. Elimination and biological half-life of mercury

Elimination of mercury from the human body varies according to the form of mercury, and elimination half-lives vary from organ to organ. Elimination of metallic mercury occurs via the urine, faeces and expired air, whilst inorganic mercury is eliminated via the urine and faeces. The main elimination route for organic mercury is via the faeces. Methylmercury is secreted into the bile where most of it undergoes entero-hepatic recycling (Clarkson, 2002).

3.1. Toxicology and elimination of mercury from the brain

The toxicology and elimination of mercury in the brain is a contentious issue. Although inorganic mercury does not itself cross the blood brain barrier in large quantities—it is found in the brain in both ethyl- and methylmercury poisoning (Magos et al., 1985) and in cases of occupational exposure to mercury vapour (Nylander et al., 1989; Opitz et al., 1996).

More controversial, however, is the question of whether methylmercury itself, or whether inorganic mercury formed by demethylation of methylmercury in the brain, forms the proximate neurotoxic agent in cases of methylmercury poisoning. Strong evidence in favour of methylmercury as the proximate toxic agent was found in a study (Magos et al., 1985) in which *Porton Wistar* rats were exposed to both ethylmercuric chloride (at 8.0 and 9.6 mgHg/kg) and methylmercuric chloride (at 8.0 mgHg/kg) via gastric gavage. Conversely, strong evidence in favour of inorganic mercury as the proximate toxic agent was found in a series of experiments carried out on *Macaca Fascicularis* monkeys exposed orally to methylmercury (at 50 $\mu\text{gHg/kg}$) (Charleston et al., 1996, 1995; Vahter et al., 1994, 1995) and is evident from human autopsy studies in cases of chronic methylmercury poisoning (Davis et al., 1994; Takeuchi et al., 1989).

At first, this evidence would seem to be contradictory. This apparent contradiction in the evidence can perhaps be resolved if we remember the age-old maxim: “The dose makes the poison”. In effect, the proximate toxic agent in either case, is most likely that form of mercury that accumulates to neurotoxic levels first. For short-term, high dose methylmercury toxicity, as used by Magos et al. (1985), the proximate toxic agent is most likely methylmercury itself due to the high dose delivered, resulting in direct toxic effects before demethylation can occur to any great extent. However for chronic, low dose exposure, as used by Charleston et al. (1996, 1995) and Vahter et al. (1994, 1995), the proximate toxic agent is most likely inorganic mercury due to both, on the one hand, its long-term accumulation in the brain and its extremely long half-life therein, and, on the other hand, methylmercury having attained a steady-state level after 1 year of exposure and failure to further accumulate within the brain whilst inorganic mercury levels continued to rise throughout the duration of the experiments (18 months).

It must also be considered that once inorganic mercury has found its way into the brain, it has a half-life therein considerably longer than that of ethyl- or methylmercury (Charleston et al., 1996, 1995; Vahter et al., 1994, 1995). As a result inorganic mercury has a tendency to accumulate in the brain in cases of methylmercury poisoning after levels of methylmercury have reached a steady state (Vahter et al., 1994). Indeed, numerous autopsy studies in cases of both mercury vapour poisoning and methylmercury poisoning have found inorganic mercury deposits in the brain many years after the cessation of exposure (Davis et al., 1994;

Hargreaves et al., 1988; Nylander et al., 1989; Opitz et al., 1996; Takeuchi et al., 1989).

Academic debate surrounding these issues is likely to continue. Nevertheless, given the available evidence indicating the selective retention of inorganic mercury in the brain, both after exposure to methylmercury via the oral route and after exposure to elemental mercury vapour, and the fact that these are the two main routes of exposure to mercury in the general population (methylmercury through the consumption of fish and mercury vapour released from dental amalgam), it would seem evident that accumulation of inorganic mercury in the brain from chronic sub-clinical exposure over long periods of time, regardless of the original form or forms of mercury to which an individual is exposed, must be considered a potential source of neurotoxicity in humans.

4. Transport mechanisms of mercury in the body

It has been known since at least the early 1970s, that 99% of mercury species circulating in the plasma are bound to protein bound thiol groups, and it was speculated that the transport of mercury into organs and resultant organ distribution was determined by the remaining 1% of mercury bound to “diffusible thiols” (Clarkson, 1972), i.e. low molecular weight thiols that are transportable across cell membranes (Lorscheider et al., 1995). More recently, in May 2005 Bridges and Zalups (2005) published a review collating various examples of endogenous thiols facilitating heavy metal transport. Their review focuses on the phenomenon of “Molecular Mimicry” and cites numerous examples where low molecular weight thiols bound to mercury (and other heavy metals) have facilitated entry of the mercury into various cell types via molecular mimicry. “Molecular mimicry refers to the phenomenon whereby the bonding of metal ions to nucleophilic groups on certain biomolecules results in the formation of organo-metal complexes that can behave or serve as a structural and/or functional homolog of other endogenous biomolecules or the molecule to which the metal ion has bonded” (Bridges and Zalups, 2005).

It seems likely that the role of molecular mimicry in heavy metal transport as summarised by Bridges and Zalups (2005), may prove highly significant clinically by elucidating the mechanisms by which toxic heavy metals are transported into differing cell types throughout the body. It is also worth noting that perhaps many more examples of “Molecular Mimicry” mechanisms remain to be discovered. Indeed, Zalups and Ahmad (2005a,b) have since reported further results that show *N*-acetyl-cysteine (NAC) conjugates of inorganic mer-

cury and methylmercury, and homocysteine conjugates of methylmercury can act as transportable substrates at hOAT1 transporters (hOAT: human Organic Anion Transporter).

5. Chelation therapy

Chelating agents are used pharmacologically in the treatment of heavy metal toxicity. Chelators are molecules that tightly bind in a ring structure to metals. A good clinical chelator will have low toxicity, will preferentially bind to the heavy metal with a high stability constant and will have a higher excretion rate than endogenous binding species for the metal, thus favouring fast elimination of the toxic metal. DMPS and DMSA are dithiol chelating agents used in the treatment of mercury toxicity. DMPS is not currently approved by the FDA for any clinical use, although it is being used to treat mercury toxicity in an off-label capacity (Risher and Amler, 2005). DMSA is approved for pediatric use in treating lead toxicity (Risher and Amler, 2005).

5.1. DMPS (*Dimaval, Unithiol*)—sodium 2,3-dimercaptopropanesulfate

DMPS was approved as a drug by the Soviet Union in 1958, but was not available in the West until about 1978 (Aposhian et al., 1995). DMPS is a water-soluble dithiol. It has been used in the treatment of arsenic, lead, mercury and cadmium poisoning and has also been used to treat Wilson’s disease (an inborn error of copper metabolism leading to bioaccumulation of copper). DMPS can be delivered orally or intravenously. DMPS is biotransformed in humans to acyclic and cyclic disulfides (Aposhian et al., 1995). It had previously been assumed that DMPS bonded to mercury in a 1:1 ratio, however X-ray absorption spectrometry studies have demonstrated that such a structure is not possible (George et al., 2004). The authors found that more complex structures must be formed by invoking at least two DMPS molecules and two mercury atoms. DMPS is not effective at removing mercury from the brain (Aposhian et al., 2003; Buchet and Lauwerys, 1989; George et al., 2004). DMPS is known to chelate the essential minerals copper, chromium and zinc (Risher and Amler, 2005).

5.2. DMSA (*Succimer, Chemet, Captomer*)—*meso*-2,3-dimercaptosuccinic acid

DMSA is administered orally and is rapidly but incompletely absorbed. It has been used to chelate lead,

arsenic, cadmium, mercury and other metals. It is rapidly and extensively metabolised and is excreted mainly *via* urine and in small amounts *via* bile and the lungs. Over 95% of DMSA in blood is protein bound (mainly to albumin) and over 90% of DMSA excreted in urine is in the form of a mixed disulfide with L-cysteine (Aposhian et al., 1995). Like DMPS, it had been thought that DMSA bonded in a 1:1 ratio with mercury. However, George et al. (2004) again found that this could not be the case. Instead they found that DMSA forms predominantly the binuclear complex $\text{Hg}_2(\text{DMSA})_2$ *in vitro*. DMSA does not effectively chelate mercury in the brain (Aposhian et al., 2003; Buchet and Lauwerys, 1989; George et al., 2004). The side-effects of DMSA include GI disorders, skin rashes and flu-like symptoms. Mild to moderate neutropenia has been reported in some patients and regular full blood counts are recommended during therapy. Renal and hepatic function should be checked before starting treatment (Sweetman, 2002). It is considered to be the least toxic of the dimercapto chelating agents (Aposhian et al., 1995). DMSA has a half-life of 3.2 h (Aposhian et al., 1992b; Frumkin et al., 2001) and is known to chelate the essential minerals copper and zinc (Risher and Amler, 2005).

6. Alpha-lipoic acid—a role in the treatment of mercury toxicity?

6.1. ALA—(Thioctic acid) alpha-lipoic acid

Alpha-lipoic acid (ALA) is a disulfide that is well known as a powerful antioxidant and is commonly available as a nutritional supplement. It is reduced intracellularly to dihydrolipoic acid (DHLA), a dithiol that has antioxidant properties. DHLA can then be exported from cells to the extracellular space. Both ALA and DHLA are known to form chelates with various heavy metals (Packer et al., 1997, 1995). Administration of ALA is known to increase intracellular GSH levels by 30–70% (Packer et al., 1997), and is known to regenerate other antioxidants such as vitamins C and E. Unlike DMSA and DMPS, ALA is taken up by all areas of the CNS and peripheral nerves (Packer et al., 1997).

ALA has been shown to be protective against the effects of acute mercury poisoning in several mammalian species when administered simultaneously or shortly after mercury exposure (Donatelli, 1955; Grunert, 1960), provided a correct dosing of ALA was used (inappropriate doses were seen to increase toxicity). Grunert (1960) suggested that frequent treatments with lower doses of ALA could also be effective by virtue of keeping the blood level of ALA more constant, and this has

been observed in guinea pigs (administered every 4 h) (Donatelli, 1955).

Aposhian et al. (2003) found that ALA administered alone or with DMSA did not chelate mercury in kidney or brain when administered to rats exposed to repeated doses of mercury vapour. However, Gregus et al. (1992) showed that administration of ALA to rats led to a greatly increased biliary excretion of inorganic mercury (12- to 37-fold). The same effect was not observed for methylmercury. Gregus et al. (1992) proposed that the inorganic mercury may have been excreted in the form of DHLA– Hg^{2+} complexes.

Further research is needed into the actions of ALA as a chelator—in particular investigation of frequent low dose chelation as suggested by Cutler (1999). Although not a peer reviewed publication, Cutler makes a plausible argument regarding the frequency of chelator dosing which merits the attention of the scientific community. Whilst it would appear that ALA may have potential as a mercury chelator, it is also clear from the work of Donatelli (1955) and Grunert (1960) that the effect of ALA on mercury toxicity is dependent on dosage size and on the spacing of dosages in time.

7. Ligand interactions and nutritional factors affecting mercury toxicity

There is a paucity of data regarding the influence that nutritional factors can have on protecting against or enhancing mercury toxicity mediated through ligand interactions and ligand competition. Given the role endogenous thiols, such as cysteine, play in transporting mercury around the body as summarised by Bridges and Zalups (2005), it would seem likely that varying levels of plasma thiols would lead to varying levels of mercury uptake by the organs. Indeed, in one study NAC supplementation appeared to increase brain mercury concentration (Aposhian et al., 2003). This raises the question as to whether dietary intake (or vitamin supplementation) of thiol-containing foods affects the transport of mercury into organs, and thus affects toxicity. Recent results have demonstrated that in rats, thiol status is an important factor in the distribution and elimination of inorganic mercury (Zalups and Lash, 2006). It has also been suggested that in humans, control of plasma cysteine levels is important in the control of symptoms and the treatment of mercury toxicity (Cutler, 1999).

7.1. N-Acetyl-cysteine (NAC)/glutathione (GSH)

NAC and GSH merit special mention as some physicians recommend them as antidotes to mercury toxicity.

At first glance this would seem logical, since GSH is known to be involved in the biliary excretion of methylmercury (Ballatori and Clarkson, 1985) and it is thought that intracellular GSH plays a role in protecting cells (Clarkson, 2002). However, only 1% of the body burden of methylmercury per day is eliminated from the gut by undergoing demethylation by microflora—the remainder is reabsorbed and undergoes entero-hepatic cycling (Clarkson, 2002). Furthermore, it has been found in rats that mercuric conjugates of GSH are in fact part of the uptake mechanism of inorganic mercury by the kidneys (Bridges and Zalups, 2005). The mercury–GSH conjugates appear to be converted to mercuric conjugates of cysteine by the enzymes γ -glutamyltransferase and cysteinylglycinase in the proximal tubules of the kidney, leading to increased uptake by the kidneys of mercury. It has also been shown that the renal uptake of methylmercury is dependent upon GSH (Richardson and Murphy, 1975). Aposhian et al. (2003) demonstrated in rats exposed to elemental mercury that NAC apparently increased brain mercury concentration. In addition, recently published results by Zalups and Ahmad (2005b) have shown that NAC conjugates of methylmercury and inorganic mercury are potentially transportable mercuric species taken up *in vivo* in proximal tubular epithelial cells. Furthermore, this latest experiment was carried out using canine Madin-Darby Canine kidney Cells (MDCK) cells transfected with the *human* organic anion transporter1 (hOAT1).

Given the inefficiency of elimination of methylmercury *via* the bile, the known entero-hepatic cycling of methylmercury and the mercury uptake mechanisms of the kidneys and the brain (Bridges and Zalups, 2005; Kerper et al., 1992) (for mercury species complexed with low molecular weight thiols), NAC and GSH would appear to be poor treatment choices for mercury toxicity due to the high risk of redistribution of mercury to those organs.

7.2. Zinc

Zinc has been seen in animal models to induce production of metallothionein, a metal binding protein, in the kidneys (Goyer et al., 1995). Metallothionein is a low molecular weight metal binding protein with a high content of cystinyl residues and metals. Mercury is known to form a complex with metallothionein and metallothionein has been shown to be neuroprotective on exposure to mercury vapour (Yoshida et al., 2005). Inorganic and elemental mercury induce metallothionein in the kidney, however methylmercury does not directly induce metal-

lothionein, but does so upon metabolism to inorganic mercury.

7.3. Selenium

Selenium is known to affect the distribution of mercury and also to reduce toxicity induced by mercury in experimental animals (Goyer et al., 1995). Intriguingly, Hol et al. (2001) reported that blood selenium levels were significantly lower in subjects who claimed symptoms of “mercury amalgam illness” than in healthy subjects with amalgam.

There is evidence that selenium in plasma forms a complex with inorganic mercury, which then binds to selenoprotein-P (Gailer et al., 2000; Sasakura and Suzuki, 1998), which in turn seems to prevent mercury uptake by the kidneys (Yamamoto, 1985). The function of selenoprotein-P is not well understood, however it is worth noting that researchers in the area speculate about it possibly playing three separate roles: (1) antioxidant defence; (2) a role in the transport of selenium; (3) a protective role as a natural heavy metal chelator (Chen and Berry, 2003).

However, it has been observed in rats that simultaneous administration of selenium (in the form of sodium selenite) and a chelating agent (DMSA or DMPS) leads to reduced excretion and considerable redistribution of mercury - specifically a reduction of kidney mercury and an increase in liver concentrations although it should be noted other organs were not examined in this study (Juresa et al., 2005). Since the chelators used (DMSA and DMPS) act to increase urinary excretion of mercury and since selenoprotein-P seems to prevent uptake of mercury by the kidneys, Juresa et al. (2005) proposed that ligand competition between the chelators and selenoprotein-P led to the redistribution of mercury and decreased urinary excretion.

A further complicating factor in the relationship between selenium and mercury toxicity, is that reduced selenium levels have been seen to induce hepatic production of GSH (Hill and Burk, 1985), resulting in a doubling of plasma GSH level. As noted earlier, GSH is involved in mercury uptake by the kidneys, so the effect of selenium on GSH levels may also be relevant to the toxic effects of mercury.

It is noteworthy that the form of selenium intake in humans is also relevant. Selenium in the form of selenomethionine has been shown to have approximately twice the bioavailability of sodium selenite and resulted in higher levels of selenoprotein-P and total selenium in plasma (Xia et al., 2005) (*note*: total selenium comprises protein-bound selenium and selenomethionine).

Clearly, the interaction between mercury, selenium, zinc and thiols is quite complex. It is likely that dietary or supplementary intake of selenium, zinc and thiol has an important role to play in mercury's effect on the body, and in the body's excretion of mercury. Further research into the influence of nutritional factors on mercury toxicity is needed.

7.4. Dietary fibre

Information on the influence of dietary fibre on mercury toxicity is lacking. However, *in vitro* studies have shown that wheat bran can effectively bind mercury and other heavy metals (Ou et al., 1999). In mice exposed to methylmercury, a 30% bran diet was seen to increase the rate of elimination of mercury from the body and to reduce the level of mercury present in the brain (Rowland et al., 1986). It has also been reported that apple pectin led to a reduced period of intoxication in children with increased urinary excretion of mercury (Sobolev et al., 1999).

This author proposes a potential mechanism of action by which increased dietary fibre may increase the elimination of methylmercury. Methylmercury is known to undergo extensive entero-hepatic recirculation (Clarkson, 2002). As fibre has been shown *in vitro* to bind mercury and as fibre is non-absorbable, it is proposed that fibre in the diet interrupts the entero-hepatic recirculation by binding the mercury and undergoing excretion, thus leading to an increased rate of elimination of mercury.

Furthermore, Gregus et al. (1992) have suggested that alpha-lipoic acid leads to increased biliary excretion of inorganic mercury in the form of DHLA–Hg²⁺ complexes. As these complexes are organic-like mercury species, it is worth considering that these complexes may be reabsorbed by the intestine with high efficiency similar to organic mercury species such as methylmercury. If this were the case, and if fibre can bind these complexes, an increased fibre intake may potentially lead to reduced reabsorption of these complexes, which would lead to improved treatment efficacy and reduced side-effects.

8. Diagnostic tests for mercury poisoning and the importance of thiols, dithiols and interacting ligands

8.1. Blood and urine levels

For recent acute exposure to mercury, blood and urine levels of mercury can be useful for diagnostic and dosage estimation purposes (Clarkson, 2002; Risher and Dewoskin, 1999; Risher and Amler, 2005). However, for

historical, chronic or low dose exposures (Risher and Dewoskin, 1999), blood and urine levels do not accurately reflect exposure levels. In addition, the retention time of mercury in certain organs, particularly the brain (Braunwald et al., 2001; Hargreaves et al., 1988; Opitz et al., 1996; Takeuchi et al., 1989; Vahter et al., 1994), is much longer than that of blood. A case worth noting is that of an industrial worker exposed to quite high levels of mercury (Opitz et al., 1996), who after treatment, showed blood and urine levels consistent with total body clearance of mercury within 3 years. However upon the patient's death, 17 years later, considerable deposits of mercury still remained in the patient's brain. Clearly in this case, blood and urine levels were not indicative of mercury body load. (Note: For urinary measurements of mercury, urinary creatinine measurements should be carried out simultaneously to control for the effects of hydration.)

9. Chelation challenge tests

In a chelation challenge test, baseline urine metal levels (usually one specific metal, e.g. lead, mercury) are measured before administration of a chelating agent (usually a bolus), and then after a given time interval a second urine sample is taken and urine mercury levels again measured. The pre-challenge and post-challenge metal levels are then compared with each other and against references.

Both DMPS and DMSA have been used to perform chelation challenge tests for mercury with mixed results (Aposhian et al., 1992a; Frumkin et al., 2001; Roels et al., 1991). Whilst some authors have chosen to focus on the clinical use of challenge tests and interpretation of results in explaining the lack of clarity in these results (Risher and Amler, 2005), it is clear that there are mechanisms and assumptions more fundamental to the methodologies of the tests themselves to be considered.

Firstly, as discussed previously, it is likely that thiol, selenium and possibly zinc levels have an effect (directly or indirectly) on the distribution of mercury. Little is known about the interaction of these species with the chelating drugs DMSA or DMPS, although it has been seen that simultaneous administration of selenium with DMSA or DMPS leads to reduced effectiveness of the chelators (Juresa et al., 2005). Current chelation challenge tests do not take any measures to control or take account of these confounding variables.

In a landmark paper on DMPS challenge tests, Aposhian et al. (1992a) found a "... highly significant positive correlation between the mercury excreted in the urine 2 h after DMPS administration and the den-

tal amalgam scores.” It is noteworthy that strict dietary controls were in place during this experiment, although this was not explicitly detailed until a later review paper was published (Aposhian et al., 1995). Clinically, chelation challenge tests are often carried out by patients at home (Risher and Amler, 2005), thus implying that standardised dietary controls are not in place. It seems plausible that the strict dietary controls used by Aposhian et al. (1992a,1995) may somewhat have minimised (or standardised) the levels of competitive ligands in the experimental participant’s plasma, leading to a clearer result.

Secondly, the large boluses that are often used clinically in chelation challenge tests carry with them an inherent risk of redistribution of mercury. As we have seen earlier, chelators are in competition with other ligands including endogenous free thiols, protein-bound thiols and metalloproteins such as selenoprotein-P and metallothionein. Redistribution has been reported in rats, purportedly due to competition from selenoprotein-P, upon administration of both DMPS and DMSA (Juresa et al., 2005). By using a large bolus, a larger amount of mercury is likely to be mobilised, thus increasing, in the event of redistribution, the quantity of mercury redistributed to target organs. Redistribution to the brain must be considered the worst-case scenario given its long half-life therein (Braunwald et al., 2001; Hargreaves et al., 1988; Opitz et al., 1996; Takeuchi et al., 1989; Vahter et al., 1994), and the inability of DMSA or DMPS to remove it from the brain (Aposhian et al., 2003; Buchet and Lauwerys, 1989; George et al., 2004). Furthermore, it must be considered that adverse drug reactions may occur, and in such an event large doses may lead to a worse reaction.

Thirdly, chelation challenge tests are often carried out whilst patients have dental amalgams in place. This raises the question of whether the chelating agents might chelate mercury from the dental amalgams leading to inaccurate results and more seriously risking an increase in the patient’s body load of mercury. This author is currently unaware of any studies investigating this possibility.

Fourthly, since DMPS and DMSA do not chelate mercury in the brain (Aposhian et al., 2003; Buchet and Lauwerys, 1989; George et al., 2004) chelation challenge tests based on these agents do not accurately reflect mercury levels in the brain. Given that the brain is one of the main target organs for both elemental and organic mercury, and that the brain retains mercury for many years (Braunwald et al., 2001; Hargreaves et al., 1988; Opitz et al., 1996; Takeuchi et al., 1989; Vahter et al., 1994), this is a fairly serious flaw.

Fifthly, there are no established reference ranges for mercury exposure and no established “safe” exposure levels to mercury (Berlin, 2003; Risher and Amler, 2005). This means results from chelation challenge tests cannot be compared to population norms, and this has been used to criticise challenge tests (Risher and Amler, 2005). There is a certain circular logic here however, since in order to establish population norms one must first establish an accurate test. Furthermore, considering that mercury is a highly toxic element with no known nutritional function, is ubiquitous in the environment (Clarkson et al., 2003), has no firmly established safe limit of exposure (Berlin, 2003; Risher and Amler, 2005) and that there is currently no widely-accepted accurate method of determining mercurial body load short of autopsy, the concept of established population norms for mercury exposure would appear, at the time of writing, to be an entirely frivolous notion.

10. Conclusions

The role of mercury in various chronic conditions such as amyotrophic lateral sclerosis, autism, Alzheimer’s disease, multiple sclerosis and Parkinson’s disease remains controversial. It is clear that there are still considerable gaps in knowledge surrounding the biological actions of mercury species in the body. It seems, however, that patients with these diseases are making their own minds up and seeking chelation treatment on their own initiative or on advice from practitioners (Berlin, 2003; Risher and Amler, 2005). Clearly, there is urgent need for further research in several key areas.

DMPS and DMSA are the drugs of choice in the treatment of mercury poisoning. Evidence suggest that they are not maximally effective in their role as chelators (George et al., 2004) and that they are ineffective at chelating mercury from the brain (Aposhian et al., 2003; Buchet and Lauwerys, 1989; George et al., 2004). Though less toxic than older chelation agents such as British Anti-Lewisite (BAL) and D-Penicillamine, they do have some toxic side-effects (particularly DMPS). There is a clear need for the development of more effective, safe chelating agents that are capable of removing mercury from the brain.

Currently, ALA represents the only potential chelating agent capable of accessing the central and peripheral nervous system. Though one particular dosing schedule was shown not to have chelating actions (Aposhian et al., 2003), previous research has shown that its actions are dependent on both dose size and timing (Donatelli, 1955; Grunert, 1960). Further research is needed to investigate the usefulness of ALA as a clinical chelator.

It seems apparent from the body of research reviewed by Bridges and Zalups (2005), that endogenous thiols, such as cysteine, homocysteine, GSH and NAC play an important role in the distribution of mercury throughout the body. This is likely to be highly relevant clinically and studies are needed to investigate the potential effect of thiol intake in both dietary and supplementary form on mercury distribution and toxicity. Many doctors advise GSH or NAC in the treatment of mercury poisoning—this does not seem advisable in light of available evidence.

Zinc and selenium would also seem to be capable of affecting mercury distribution and protecting against toxicity. These would seem to be highly dynamic relationships and to be poorly understood at present. Other essential metals may also be important and the interaction of zinc and selenium with chelators such as DMPS/DMSA are poorly characterised.

The effect of dietary fibre on the distribution and elimination of mercury remains a largely unexplored area of research. However, the handful of studies that do exist seem to point to dietary fibre as potentially enhancing the elimination of methylmercury from the body. The effect of dietary fibre on the elimination of DHLA–Hg²⁺ remains unquantified.

There is an urgent need for an accurate method of testing for mercury levels in clinical practice for cases of historical, chronic or low dose exposure to mercury. Whilst blood and urine measurements are recommended for diagnosis of mercury poisoning (Risher and Amler, 2005), these are clearly only useful for recent acute exposures, are of little use for measuring historical, chronic or low dose exposure, and are not indicative of brain mercury levels. Current chelation challenge tests are inaccurate as used clinically, and owing to the large doses often used, carry risks to the patient of redistribution of mercury and risks due to adverse drug reactions. It is also unclear what effect chelators will have on a patient's existing dental amalgam fillings.

Population exposure norms for mercury body load, and a safe exposure level have not been established for mercury. In the absence of accurate clinical testing such concepts have little meaning in any case. Furthermore, whilst debate rages over the safety of dental amalgam, thimerosal and the consumption of mercury containing fish and the possible role of mercury in chronic diseases, logically it would seem resolution of these issues is impossible without first establishing an accurate clinical testing method for determining mercurial body load in supposedly affected individuals.

Given the possibility that mercury could play an important role in various chronic diseases, the mys-

teries surrounding mercury are ones that need urgent resolution. It is becoming apparent that thiols, dithiols, nutritional factors and ligand interactions play an important role in the toxicology of mercury. An improved understanding of the role these molecules play would appear to be key to improved clinical testing for mercury toxicity and perhaps for developing more effective treatment protocols for victims of mercury poisoning.

Conflict of interest statement

No conflicts of interest exist.

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