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REVIEW ARTICLE

Chemical and biological properties of toxic metals and use of chelating agents for the pharmacological treatment of metal poisoning

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Abstract Exposure to toxic metals is a well-known problem in industrialized countries. Metals interfere with a number of physiological processes, including central nervous system (CNS), haematopoietic, hepatic and renal functions. In the evaluation of the toxicity of a particular metal it is crucial to consider many parameters: chemical forms (elemental, organic or inorganic), binding capability, presence of specific proteins that selectively bind metals, etc. Medical treatment of acute and chronic metal toxicity is provided by chelating agents, namely organic compounds capable of interacting with metal ions to form structures called chelates. The present review attempts to provide updated information about the mechanisms, the cellular targets and the effects of toxic metals.

Keywords Metal poisoning · Lead · Cadmium · Mercury · Arsenic · Chromium · Nickel · Chelating agents

Introduction

Exposure to potentially toxic metals represents a widespread problem in most industrialized countries. In fact, although metals occur naturally in the ecosystem, anthropogenic sources, i.e. pollution, contribute to their introduction in the ecosystem. Toxic metals generally interfere with a number physiological processes, including central nervous system (CNS), haematopoietic, hepatic and renal functions.

A generally accepted classification of metals is based on their role in living organism. Thus, "essential metals", such as copper, iron, magnesium and zinc, are those indispensable for several biological processes, functioning as enzymatic cofactors or as functional groups of proteins (e.g., iron in haemoglobin). By contrast, "non essential metals", such as arsenic, cadmium, lead, mercury and chromium, do not play any role in physiological functions and are often considered as toxicants. Nevertheless, this classification is not absolutely correct, since both classes of metals may potentially disturb normal biological functions, being their toxicity concentration dependent. Indeed, short supply of essential metals can affect proper functioning of many tissues and organs, whereas an excess of essential metals can provide toxicity through mechanisms similar to those ascribed to non essential elements.

Metals and semimetals (metalloids) associated with contamination and potential toxicity or ecotoxicity are often defined "heavy metals". This designation refers to an extremely disparate group of elements, and even a more disparate group of their compounds, including elements lighter than carbon and excluding some of the heaviest metals that often lack functional similarities in their chemical, biological and toxicological properties. Therefore, the term "heavy metals" has been queried for many years and efforts to replace it by chemically sound terminology have so far failed (see Duffus 2002). In general, scientific literature considers as heavy metals the following elements: aluminium, iron, silver, barium, beryllium, cadmium, cobalt, manganese, mercury, molybdenum, nickel, lead, copper, tin,

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titanium, thallium, vanadium, zinc and some metalloids, such as arsenic, bismuth and selenium, with properties similar to heavy metals. Most heavy metals are characterized by atomic density and number higher than 5.0 g/cm^3 and 20, respectively, low water solubility of their hydrates and high tendency to form complex compounds. Several toxic metals possess high affinity for thiolic, aminic, phosphoric and carboxylic groups of organic compounds, thus showing a high tendency to combine with reactive sites of the biological molecules, including proteins and nucleic acids.

Heavy metals are considered among the most dangerous and damaging polluting substances. They may be found in food, water and air, and, when high amounts are assumed, they may alter biological functions and cause damage. In the last century, their mobilization due to the technological progress linked to human activities (agriculture, industry, combustion processes) and their consequent introduction into the environment as polluting waste have promoted their growing accumulation in the biosphere and their introduction into the food chain with consequent serious health risks for humans, animals and plants.

In particular, the metals of the Earth crust can enter the environment through geologic, biologic and anthropogenic processes. Natural sources of metals are erosions, volcanic eruptions, wood fire and bioaccumulation due to their introduction into the food chain though plants and animals. The most important anthropogenic sources of environmental metal pollution include combustion of fossil fuels, foundry, mining and manufacturing industries, as well as civil and industrial waste disposal. Obviously, atmospheric contamination in industrial areas is mainly due to anthropogenic causes when compared to natural sources.

Factors affecting metal toxicity

Several factors have to be taken into account when considering toxicity of metals. In general, children and elderly persons are more susceptible than adults to the deleterious effects of metals. In fact, children are at higher risk of metal exposure through food, since they need more calories per kilogram of body weight and have a higher gastrointestinal absorption of metals when compared to adults (Heath et al. 2003). For some metals, exposure to an excessive amount is well tolerated, since absorption is limited to the amount required by the individual; whereas, others may show a strong tendency to accumulate. For instance, the half-life of mercury is 60–70 days, whereas that of cadmium is 10–20 years. Moreover, half-life may vary with the type of tissue where the metal accumulates. Accordingly, half-life of lead in soft tissues corresponds to some weeks, whereas in the bone it may reach 20 years.

Two important parameters that have to be taken into account when considering the toxicity of a particular metal are its chemical forms (elemental, organic or inorganic) and its binding capability. In fact, the chemical form can strongly affect the pharmacokinetic properties of the metal, including its absorption, distribution and ability to reach the cellular and intracellular targets.

Organic forms of metals are usually highly lipophilic and, thus, easily cross biological membranes (e.g., gastrointestinal wall, placenta, blood–brain barrier). Accordingly, the organic forms of mercury induce neurotoxicity, due to their ability to cross the blood–brain barrier and to accumulate in lipophilic tissue, whereas the inorganic forms of the metal mainly cause renal toxicity.

In some cases, toxicity of non essential metals can be ascribed to their ability to compete, in virtue of physical–chemical similarities, with essential metals, thus disrupting homeostatic ionic equilibrium. This is the case of lead and cadmium that may interact with calcium and iron equilibrium. For example, lead can substitute for iron in the ferrochelatase structure, thus disrupting iron incorporation into haemoglobin (Fig. 1). Inhibition of ferrochelatase by lead represents one of the mechanisms implicated in the development of anaemia (Labbé et al. 1999; Rettmer et al. 1999). Moreover, gastrointestinal absorption of lead is increased in conditions of lack of iron (Bradman et al. 2001). Lead, in fact, competes with iron for the binding to intestinal ferritin. Similarly, in conditions of short calcium supply, stimulation of the synthesis of proteins implicated in calcium binding at the gastrointestinal level, promotes absorption of lead and cadmium.

Metal-binding proteins

It is important to underline that toxic effects of metals are often tissue-specific, and this is in most cases due to the presence of specific proteins that selectively bind metals. The metal–protein complexes, that are usually devoid of enzymatic activity, play several roles in metals homeostasis, as they represent a form of temporary reservoir, contribute to the transport of essential metals and may play a detoxicant role by limiting an excess of free metal concentration. Typical examples of these proteins are as follows: *Calmodulin* that binds calcium, *Ferritin* and *Transferrin* that bind iron, *Ceruloplasmin* that is involved in copper transport, *Metallothionein* (MT) that binds copper, zinc, mercury and lead (Table 1). These proteins are particularly rich in cysteine residues that may be implicated in the interaction with the metals.

MT serves many roles in both normal and pathological conditions, acting as a reservoir of essential heavy metals (e.g., Cu^{2+}, Zn^{2+}), as a scavenger for both heavy metal

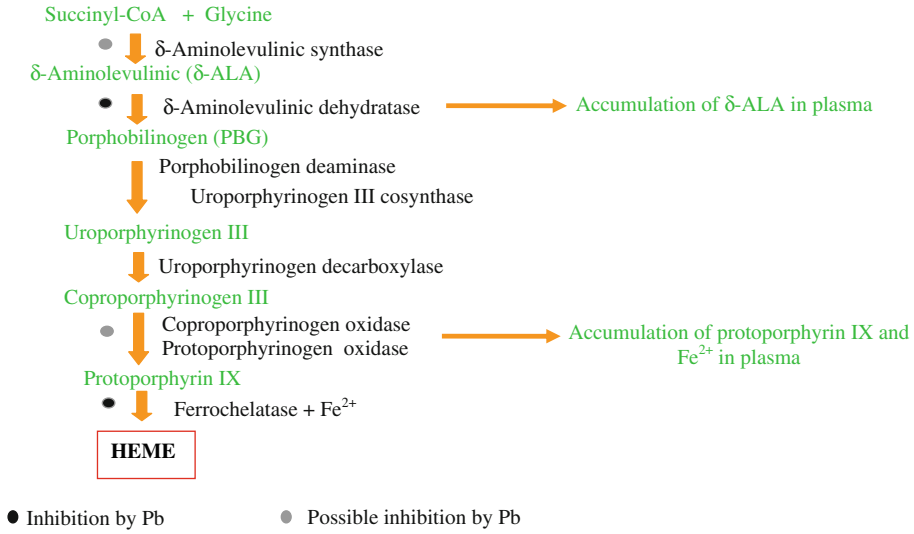

Fig. 1 Inhibition of haeme biosynthesis by lead

Table 1 Metal-binding proteins

Protein	MW (D)	Localization	Metal	Function
Calmodulin	14,000	Ubiquitous	Ca	Activator of various enzymes (second messenger)
Ferritin	470,000	Bone marrow, intestine, liver	Fe	Deposit
Transferrin	90,000	Plasma, extracellular space	Fe	Transport
Ceruloplasmin	132,000	Plasma	Cu	Transport
Metallothionein	6,500	Ubiquitous	Ag, Hg, Cu, Cd, Pb, Zn	Deposit

toxicants (e.g., Hg^{2+}, Cd^{2+}) and free radicals, and as a regulator of transcription factor activity (Vasak 2005). In mammals, two isoforms of MT have been isolated, which are expressed in most organs (MT-I and MT-II), and a isomeric form (MT-III), mainly expressed in cerebral tissue. Recent studies have underscored the crucial involvement of MT in the modulation of the immune system by metals (Lynes et al. 2007).

Hypersensitivity reactions to metals

Some metals (e.g., Hg, Au, Pt, Be, Cr, Ni) may induce hypersensitivity reactions, and in such cases it is necessary to evaluate the immune reactivity of the subject exposed. Heavy metals behave as haptens, since they are devoid of antigenicity, but become fully antigenic when associated with proteins (Büdinger and Hertl 2000; Martin et al. 2006).

Metals can potentially induce all the four forms of hypersensitivity reactions (Fig. 2):

- Type I: this reaction can be caused, for example, by platinum exposure, is characterized by IgE production and is responsible for asthma, urticaria and anaphylaxis;
- Type II: this reaction can be caused, for example, by exposure to organic salts of gold. It is mainly mediated by IgG and is often associated with thrombocytopaenia.
- Type III: in addition to gold, this reaction can also be triggered by exposure to mercury vapours. It is characterized by the formation of immune complexes that precipitate and cause damage to the glomerulus and, thus, proteinuria.
- Type IV: chromium and nickel exposure are possible causes of this type of reaction. It is a delayed-type hypersensitivity reaction, characterized by a cell-mediated, antibody-independent, immune memory response that causes contact dermatitis (e.g. after Cr or Ni exposure) or formation of granulomas (e.g., after Be or Zr exposure).

Carcinogenicity

Having a high affinity for nucleophilic centres of nucleic acids, most metals may function as carcinogens or co-carcinogens (Salnikow and Zhitkovich 2008; Snow 1992). Nevertheless, carcinogenic metals are typically weak mutagens and, with the exception of chromium, they do not form DNA adducts, which represent a pivotal initiating event in cancer-inducing activity of organic carcinogens.

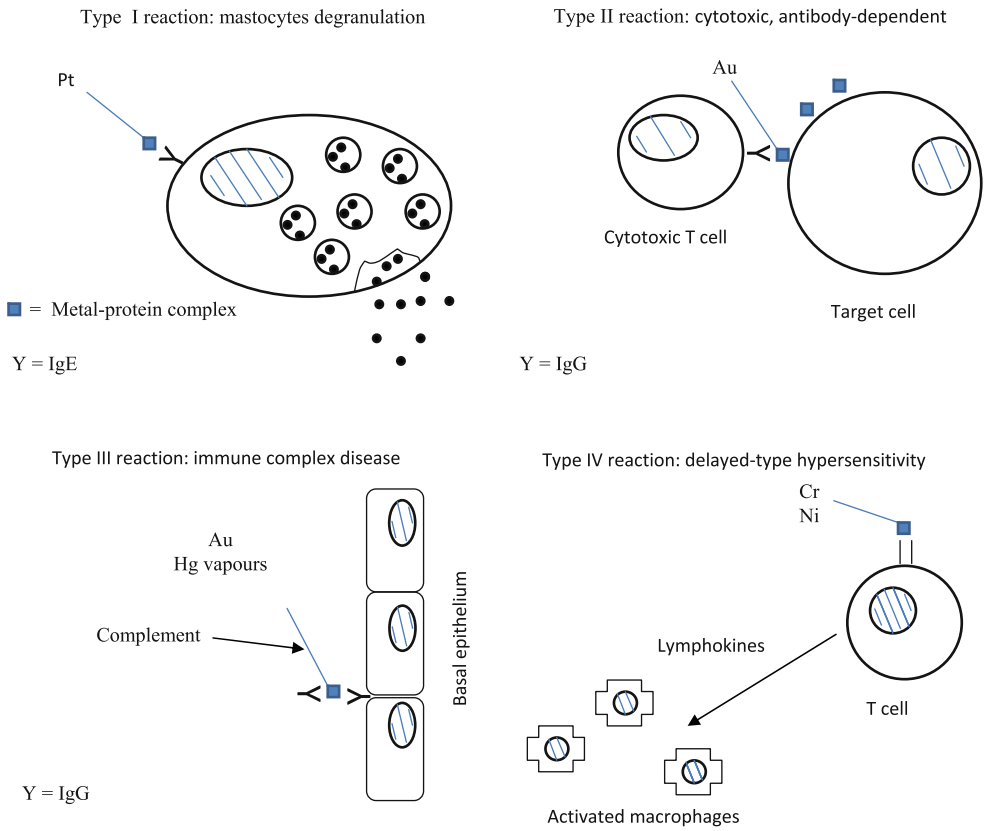

Fig. 2 Hypersensitivity reactions to metal ions

The exact mechanism implicated in the genotoxic action of metals is not completely understood, although several studies have suggested the existence of a correlation between cancerogenicity, electronegativity and solubility. Usually, the cancerogenic potency increases with increasing electronegativity and decreasing water solubility. The electronegativity range for most carcinogenic metals is between 1.2 and 1.9. Moreover, the scarcely soluble oxides and sulphides of Ni and Cr are more potent carcinogens than are the soluble salts.

Based on epidemiological evidence, arsenic, cadmium, chromium (VI) and nickel compounds are classified as human carcinogens, and there is evidence suggesting that inorganic lead compounds, metallic nickel and its alloys may also be carcinogenic in humans (Table 2). By contrast, the cancerogenicity of cobalt, iron, manganese, platinum, titanium and zinc has also been confirmed in animal studies and at very high doses.

Table 2 IARC classification of metals and/or their compounds as human carcinogens

Substances	IARC category
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Arsenic and arsenic compounds	Group 1
Arsenic in drinking water	Group 1
Beryllium and beryllium compounds	Group 1
Cadmium and cadmium compounds	Group 1
Chromium [VI]	Group 1
Chromium [III]	Group 3
Chromium, metallic	Group 3
Gallium arsenide	Group 1
Lead	Group 2B
Lead compounds, inorganic	Group 2A
Lead compounds, organic	Group 3
Nickel compounds	Group 1
Nickel, metallic and alloys	Group 2B

Group 1 the agent is carcinogenic to humans, *Group 2A* the agent is probably carcinogenic to humans, *Group 2B* the agent is possibly carcinogenic to humans, *Group 3* the agent is not classifiable as to its carcinogenicity to humans

Lead

Lead is an element of the IV group of the periodic system. It is a naturally occurring element and a very common environmental contaminant. Lead is used in many industries, including lead smelting and processing, the manufacturing of batteries, pigments, solder, plastics, cable sheathing, ammunition and ceramic glazes. Moreover, tetraethyl and tetramethyl lead have been in use as anti-knock additives in

gasoline for almost 60 years. Although most cases of lead poisoning in adults result from occupational exposure, lead exposure in the general population is primarily through diet and from old lead-based paint (Brodkin et al. 2007).

Absorption of environmental lead occurs through the lungs and gastrointestinal tract for both organic and inorganic forms; organic lead compound may also be absorbed through the skin. Gastrointestinal absorption is the most common route of exposure to lead in the general population, being higher in children (40%) than in adults (5–15%). By contrast, in occupational settings, exposure is largely through inhalation (Brodkin et al. 2007). Indeed, combustion of fossil fuels containing lead may release in the atmosphere lead dioxide and lead carbonate, as well as particles of PbBrCl or gaseous Pb that can be readily absorbed through the lung.

Following absorption, lead enters the bloodstream where it is predominantly bound to erythrocyte proteins (Fig. 3), with an average clearance half-time of approximately 35 days (Rabinowitz 1991; O'Flaherty 1993). Clearance occurs through distribution into soft tissues (brain, liver, kidney, bone marrow), bone, where it accumulates as $Pb_3(PO_4)_2$, as well as excretion, primarily via the kidneys. A small amount of the metal is also excreted in faeces, sweat, hair and nails. Lead circulates widely and easily crosses the blood–brain barrier and placenta, making the brain and the developing foetus among the targets of concern (Hu 1998). Up to 95% of the body burden of lead is in bone, where it has a half-life of years to decades (Hu et al. 2007). Pregnancy, lactation, menopause, osteoporosis and other events that lead to increased bone resorption will cause an increase in blood lead levels in people who have substantial amounts of the metal stored in bone, and it can be an unexpected source of poisoning.

Given its high capacity of accumulating in bone and erythrocytes, acute intoxications by lead are rare. Symptoms include nausea, vomiting, constipation or diarrhoea, dark faeces (for the formation of PbS), abdominal pain, anorexia, hypothermia and hypotension. It may also cause peripheral neuropathy, nephropathy and anaemia.

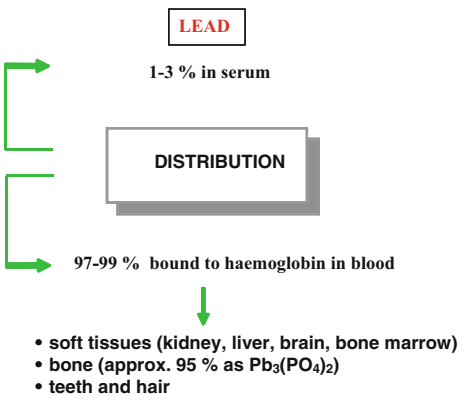

Fig. 3 Tissue distribution of lead

Chronic intoxication (also known as plumbism or saturnism) is more common and is often associated with occupational exposure (Patrick 2006). Symptoms are mainly gastrointestinal (nausea, abdominal pain), neuromuscular (loss of coordination, numbness and tingling in the extremities) and neurological (loss of short-term memory or concentration, depression, irritability, headaches), also including alterations of haematopoiesis (anaemia). Saturnism is also characterized by a "lead hue" of the skin with pallor and by a blue line along the gum (Pearce 2007), as well as by hepatic or renal complications. Chronic lead exposure can potentially induce an irreversible nephropathy that is often associated with the development of saturnine gout and hypertension and may ultimately evolve into renal failure (Nolan and Shaikh 1992). In children, lead may cause reduced growth and slowed cognitive development, with neuropsychological deficits occurring at blood lead levels lower than 10 μg/dl (Murata et al. 2009). In fact, lead can affect several neurotransmitter systems and in particular the mechanisms of synaptic plasticity implicated in learning and memory (White et al. 2007).

At a molecular level, lead binds to many proteins, especially to thiol and carboxyl groups, and mimics calcium in many biological pathways (Rabinowitz et al. 1973; Kern et al. 2000). Thus, lead can inactivate several enzymes, including those implicated in haeme biosynthesis (Fig. 1). The enzymes delta-aminolevulinic acid dehydratase that catalyses the formation of the porphobilinogen ring, and ferrochelatase that inserts iron into the protoporphyrin ring, both are compromised by lead. This, together with an increased fragility of erythrocyte cell membrane contributes to the appearance of anaemia, characterized by reduced levels of haemoglobin, haematocrit and red blood cells count.

Many toxic effects of lead also result from its inhibition of cellular functions requiring calcium. In fact, lead binds to calcium-activated proteins with much higher affinity than calcium itself, thus altering the function of a number of calcium-dependent effector mechanisms, such as calmodulin, protein kinase C, Ca^{2+}-dependent K^+ channels in the plasma membrane and neurotransmitter release.

In addition to the appearance of clinical signs and symptoms, lead poisoning should be confirmed by determination of lead concentration in blood and protoporphyrin in erythrocytes. In fact, since lead at low concentrations decreases haeme synthesis, it is diagnostically important to measure levels of δ-aminolevulinc acid and coproporphyrin in the urine and of zinc protoporphyrin in the red cells as erythrocyte protoporphyrin.

Initial treatment of acute exposure to lead involves supportive measures, including control of fluid and electrolyte

balances, diazepam or phenytoin to treat seizures, mannitol and dexamethasone to treat cerebral oedema. In symptomatic patients or in patients with a blood lead concentration in excess of 50–60 μg/dl (about 2.5 μM), chelation therapy should be performed with edetate calcium disodium ($CaNa_2EDTA$), dimercaprol, D-penicillamine and 2,3-dimercaptosuccinic acid (Bradberry and Vale 2009; Patrick 2006; Kalia and Flora 2005). $CaNa_2EDTA$ and dimercaprol are usually employed in combination for the treatment of lead encephalopathy.

Cadmium

Cadmium possesses two electrons in the outermost s subshell and a complete d subshell. This makes cadmium a post-transition metal, characterized by a low melting point, similar to mercury. Moreover, similar to zinc, the soft, bluish-white cadmium prefers the oxidation state +2 in most of its compounds.

Cadmium occurs in nature at low concentrations, mainly in association with the sulphide ores of zinc, lead and copper. In fact, cadmium ores are not abundant, but given its similarity with zinc, cadmium is found in most zinc ores as a result of isomorphic substitution. However, due to the widespread nature of its occurrence, cadmium is present in measurable amounts in almost everything we eat, drink and breath (WHO 1992a, b).

Human activities contributing to cadmium contamination of the environment include combustion of fossil fuels, leachate from landfill sites, run-off from agricultural land and mining residues, especially from zinc and lead mines (Muntau and Baudo 1992). Cadmium is also produced as a by-product in the manufacturing of Ni–Cd batteries, pigments, stabilizers and alloys and in the electroplating to protect steel from corrosion (WHO 1992a, b; IARC 1993; Martelli et al. 2006).

Atmospheric deposition of airborne cadmium, mining activities and the application of cadmium-containing fertilizers and sewage sludge on farm land may lead to the contamination of soils and increased cadmium uptake by crops and vegetables grown for human consumption.

Cadmium has been recognized as an occupational health hazard for many decades, whereas the risks to environmentally exposed populations were emphasized later (Hagino and Kono 1955). Indeed, the general population can be exposed to the health effects of cadmium mainly through ingestion of contaminated food or tobacco smoke, also in the absence of specific industrial exposure. It has been estimated that the average cadmium intake is between 8 and 25 μg per day, with more than 80% of the food-metal coming from cereals, vegetables and potato (Olsson et al. 2002). In addition to that, cadmium may derive from tobacco smoking, since a person smoking 20 cigarettes per day will absorb about 1 μg of the metal per day (Järup and Åkesson 2009). Recently, the European Food and Safety Authority (EFSA) performed a meta-analysis of a large number of studies and established a tolerable weekly intake (TWI) of 2.5 μg/kg (EFSA 2009).

The dietary cadmium absorption rate is about 5%, rising to 20–30% in some individuals. By contrast, bioavailability of inhaled cadmium oxide is relatively high, with 10% deposited in lung tissues and another 30–40% absorbed into the systemic blood circulation of smokers (Satarug and Moore 2004).

Although classified among the five more toxic metals, cadmium has been considered a "stimulatory element" or an "essential ultratrace element" for its ability to slightly stimulate growth of animals fed low (less than 5 μg/kg diet) cadmium (Nielsen 1998). In vitro, the metal has transforming growth factor activity and stimulates cell growth. Because it is consistently associated with metallothionein, cadmium may have some biochemical effects through this biosubstance (Kostial 1986). However, given its long half-life and its toxicological properties, further studies are needed to better define its biological functions and its nutritional importance at low intakes.

The biological half-life of cadmium is very long and biphasic, having a fast component of 75–128 days and a slow component of 7.4–26 years (Järup et al. 1983; Matsuno et al. 1991; WHO 1992a, b), thus raising the possibility of cumulative effects even at low-level intake.

After absorption, cadmium bound to albumin is transported to the liver, where it promotes the synthesis of metallothionein, a small cysteine-rich heavy metal–binding protein (Nordberg et al. 1992). The MT–cadmium complex is then released from the liver to the plasma and eliminated in the urine. MT-bound cadmium can be reabsorbed from the glomerular filtrate by the renal tubule cells, where it is cleaved by lysosomal action, thus releasing Cd^{2+} ions that are re-excreted into the tubular fluid (Nordberg et al. 1992) (Fig. 4).

Although the binding to MT is responsible for accumulation of the metal in tissues and for its long biological half-life in the body, induction of MT has been shown to protect against acute cadmium-induced lethality and acute toxicity to the liver and lung. Intracellular MT also plays important roles in ameliorating cadmium toxicity following chronic exposure, particularly to the kidney, bone, lung, liver and immune system (Klaassen et al. 2009).

Acute cadmium poisoning causes pulmonary oedema, haemorrhage, fulminate hepatitis, testicular injury, and lethality; whereas prolonged exposure to the metal produces nephrotoxicity, osteotoxicity and immunotoxicity (ATSDR 1999; Liu et al. 2007a, b). A wide spectrum of deleterious effects on the reproductive tissues and the

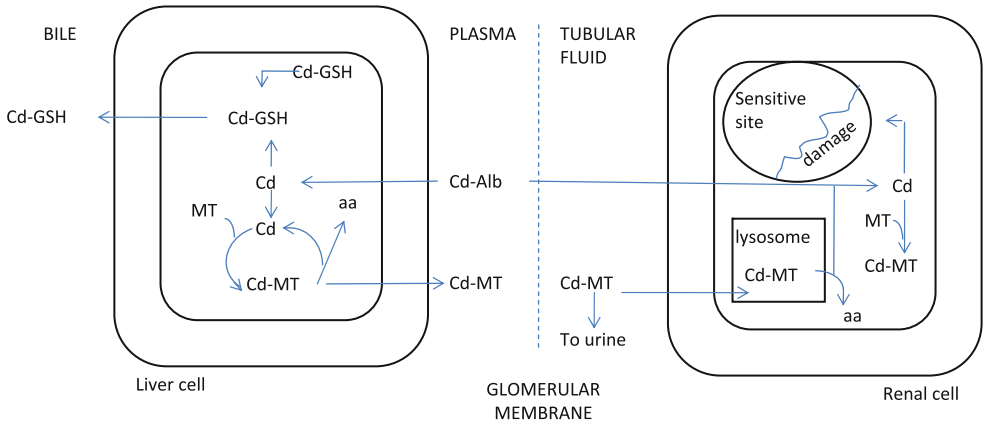

Fig. 4 In the liver, Cd can bind to glutathione (GSH) and be excreted through the bile or it may bind to metallothionein, thus forming a complex (Cd-MT) that represents a form of metal accumulation. When the Cd-MT complex is released in plasma, it can be eliminated with the urine and reaches the renal tubule cells, where it is hydrolysed by the lysosomes to MT and Cd^{2+}. Metal ions can then be re-excreted into the tubular fluid

developing embryo has also been described (Thompson and Bannigan 2008). More recently, cadmium, even at low-level environmental exposure, has been classified by IARC as a class 1 human carcinogen (IARC 1993), causing tumours to the lung, prostate, pancreas and kidney (Waalkes 2003; Järup and Åkesson 2009). In fact, although being only weakly genotoxic, cadmium may trigger adaptive, stress-induced protective and antiapoptotic mechanisms (Beyersmann and Hartwig 2008). Other cellular effects of this toxic metal are induced by disruption of physiological signal transduction systems, including those mediated by Ca^{2+}, cAMP, NO, MAP-kinase, PKB/Akt and nuclear factor-kappa-B (Thévenod 2009).

The major target organ following acute cadmium poisoning is liver, and hepatotoxicity is considered the major cause of lethality due to acute exposure (Goering and Klaassen 1983). Changes within the liver are dose- and time-dependent, ranging from moderate diffuse hepatocellular degeneration through to multifocal necrosis (El-Ashmawy and Youssef 1999; Sauer et al. 1997). Acute exposure to cadmium fumes (as CdO) or aerosols produces pulmonary oedema and haemorrhaging followed by inflammation, scarring, fibrotic changes and carcinogenesis (ATSDR 1999; Waalkes 2003). In experimental animals, there is also evidence of an acute, rapid toxicity to testis, characterized by swelling, congestion, oedema, haemorrhage and necrosis.

Following chronic dietary exposure, the major target organ is the kidney, where cadmium accumulates with a half-life of approximately 10–30 years. Renal damage is characterized by proximal tubular reabsorptive dysfunction and glomerular damage, with early increase in low molecular weight proteins (β2 and α1 microglobulins) excretion, but also glycosuria and aminoaciduria (threonine and serine) (Bernard 2008; Kobayashi et al. 2008). Moreover, cadmium may potentiate diabetes-induced effects on kidney (Åkesson et al. 2005; Buchet et al. 1990; Chen et al. 2006). For decades, it has been thought that nephrotoxicity was mediated by the cadmium–MT complex, since the latter is extremely toxic to the kidney after i.v. injection to experimental animals (Nordberg et al. 1975). Nevertheless, more recent findings have suggested that nephrotoxicity is due to accumulation of inorganic cadmium, rather than metal–MT complex (Klaassen et al. 2009).

It has been known since the 1950s that prolonged exposure to high cadmium levels may give rise to bone disease, first reported from the Jinzu river basin in Japan. In the decades leading up to World War II, Japanese mining operations contaminated the Jinzu River with cadmium and traces of other toxic metals. The local agricultural population consuming rice irrigated with the contaminated water developed the so-called Itai-Itai disease, characterized by multiple fractures and distortion of the long bones in the skeleton, and by severe pain in the joints and spine (Järup et al. 1998). The disease exhibits a mixed pattern of mainly osteomalacia but also osteoporosis in combination with kidney damage and anaemia. Bone demineralization begins soon after cadmium exposure, well before the onset of kidney injury. Cadmium exposure in conjunction with calcium deficiency, pregnancy, and lactation are key aetiologic factors for Itai-Itai disease (Wang et al. 1994). Interestingly, several studies have addressed a possible association between long-term low-level environmental cadmium exposure and osteoporosis (Bhattacharyya 2009). There is evidence that low-level cadmium exposure has negative effects on bone mineral density, produces reactive changes in calciotropic hormones and increases calciuria as a result of increased bone resorption (Åkesson et al. 2006; Schutte et al. 2008a). Cadmium alters calcium metabolism as it reduces the normal activation of vitamin D in the kidney

and binds to the intestinal calcium-binding protein, thus reducing calcium absorption from the gut and impairing bone mineralization (Washko and Cousins 1977; Berglund et al. 2000).

Urinary cadmium concentration has been associated with myocardial infarction and with changes in some physiological indicators of cardiovascular function, i.e. pulse wave velocity, arterial pulse pressures and arterial compliance and distensibility (Everett and Frithsen 2008; Schutte et al. 2008b). The pathogenesis of these abnormalities is unclear at present.

Cadmium in blood is widely used as biological indicator of current exposure, while urinary concentration is usually measured to estimate chronic exposure risks. Moreover, because of its half-life of several months, the amount of cadmium accumulated in hair should better reflect an average of integrated environmental exposure, than does blood or urine. However, the possibility of exogenous contamination has lead to substantial controversy concerning the reliability of hair analysis as a measure of the absorbed dose.

Based on data from several European studies (Buchet et al. 1990; Hotz et al. 1999; Järup et al. 2000), a recent European risk assessment report (EU RAR 2007) proposed a LOAEL of 2 μg Cd/g creatinine. However, in an evaluation of the risk assessment document, the EC Scientific Committee on Toxicity, Ecotoxicity and the Environment concluded that effects may occur even at levels as low as 0.5 μg/g creatinine (EC 2004).

Although there is no proven benefit, some clinicians recommend chelation therapy with $CaNa_2EDTA$. The dose of $CaNa_2EDTA$ is 75 mg/kg per day for 5 days. Alternatively, therapy with dimercaprol and substituted dithiocarbamates appears promising for individuals chronically exposed to cadmium.

Mercury

Mercury is a non transition metal, like zinc and cadmium. The symbol Hg derives from the latinized greek word "hydrargyrum" (meaning water or liquid silver), since this silvery metal is liquid at standard conditions of temperature and pressure. Mercury is an extremely rare element in the Earth's crust, having an average crustal abundance by mass of only 0.08 ppm. However, given its low tendency to blend with other elements that constitute the majority of the crustal mass, mercury ores can be extraordinarily concentrated, containing up to 2.5% of the metal. The most common mineral is cinnabar (HgS), which is highly toxic by ingestion or inhalation of the dusts, and can be roasted to oxide that decomposes at 500°C, releasing mercury vapours by distillation.

Compared to other heavy metals, mercury has a peculiar behaviour. In fact, it is monoatomic in vapour phase and, already at 20°C, has a relatively high vapour pressure (1.3 10^{-3} mm). It is highly soluble in both polar and non-polar liquids, i.e. a saturated solution in disareated water at 25°C contains 6.39 10^{-7} g/L of the metal. Mercury is easily released from diluted aqueous solutions and from solution of Hg(II) salts, following its reduction caused by the presence of reducing agents or by dismutation.

Given its high volatility and extended lifetime, the gaseous phase of elemental mercury (Hg^0) plays an important role in the transport of the metal in the geoclimatic systems. Mercury has a long (approx. 1 year) atmospheric residence time. The natural sources of mercury emissions to the atmosphere are represented mainly by volcanic emissions and, to a lesser extend, by volatilization of the metal from aquatic environments, re-emission from vegetation, degassing from geological materials and release associated with wind-blown dust (e.g. Lindqvist et al. 1991; Mason et al. 1994; Lamborg et al. 2002). Anthropogenic sources include burning of fossil fuels (e.g., coal-fired power plants), metal mining and extraction, cement production and disposal of products containing mercury (Pacyna et al. 2009). In fact, mercury is used in several scientific and medical apparatus, such as thermometers, barometers, manometers, float valves and sphygmomanometers, in dentistry (amalgam material for dental restoration) and in electricity (fluorescent lamp bulbs), plastic and paint industries (EPA 2009).

Vapours of atomic Hg and inorganic salts (HgO, HgS and Hg_2Cl_2) may be responsible of human poisoning, but the most toxic forms are organic compounds, such as methylmercury, ethylmercury and phenylmercury. Indeed, the chemical form of mercury strongly affects its route of exposure and bioavailability, as well as the toxicity profile (Fig. 5) (Clarkson et al. 2003; Guzzi and La Porta 2008).

The vapours of elemental (metallic) mercury, being highly liposoluble, are readily absorbed through the lungs (80%) and easily distribute in tissues and organs. Acute poisoning is characterized by damage to the liver, the kidney and the nervous system. Organic compounds are rapidly absorbed through the skin, the gastrointestinal tract or the lungs and, due to their high liposolubility, bioavailability is extremely elevated (more than 80%). Methylmercury binds (more than 90%) to erythrocytes and accumulates mainly in liver and, to a lower extent, in kidney. Toxic effects of organic derivatives include anaemia, neurological deficits and alterations of embryonal and foetal development. This latter effect is of interest since all chemical forms of mercury can cross the placenta. Inorganic salts (e.g., $HgCl_2$) are usually absorbed through the gastrointestinal tract, but display a low bioavailability (5–10%) when compared to organic compounds, and may produce inflammatory reactions in kidney or gastrointestinal apparatus.

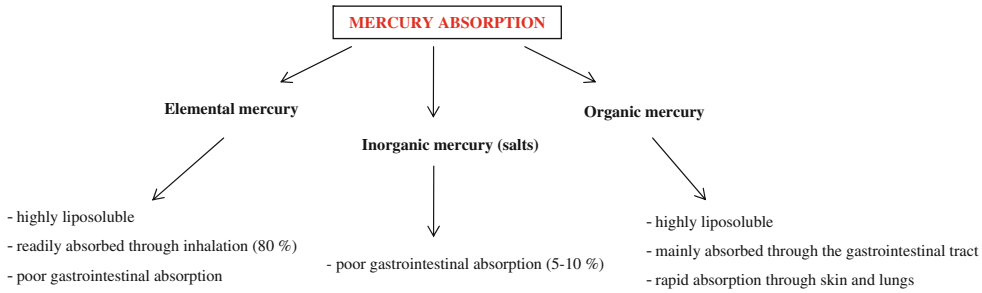

Fig. 5 Bioavailability of mercury and its compounds

The general population is primarily exposed to mercury vapour released from dental amalgams and to organic (methyl) mercury from fish consumption (Clarkson et al. 2003; DHHS 2009). In fact, in natural aquatic systems, anaerobic bacteria synthesize methylmercury from inorganic mercury as a by-product of their life processes (Ullrich et al. 2001). Methylmercury is then easily taken up by aquatic plants and animals, reaching the top of the aquatic food chain and, ultimately, humans. Other sources of exposure include Thimerosal (ethyl mercury), a preservative found in some vaccines (Bigham and Copes 2005), and some Ayurvedic medicinal products (Saper et al. 2004). Exposure risks from other sources are confined to certain sectors, including occupationally exposed workers and people using mercury-containing products.

Elimination of Hg^0 is through urine (60%) and faeces (40%, as Hg^{2+}) and its half-life in the blood, similarly to ionic mercury, is approximately 60 days. However, half-life in brain tissue is much higher due to formation of complexes with selenium that are biologically inactive. Organic mercury is mainly (90%) eliminated through the bile, after being complexed with glutathione and cysteine in the liver. Almost half of methylmercury secreted in the bile is demethylated to Hg^0 and excreted as such. Depending on the type of compounds, the half-life of organic mercury is between 40 and 105 days (Clarkson et al. 2003; Brodkin et al. 2007).

Mercury readily forms covalent bonds with sulphur atoms, and this property underlies most of its biological and toxicological actions. In particular, divalent mercury can replace the hydrogen atom in sulphydryl groups to form mercaptides, X-Hg-SR and $Hg(SR)_2$, where X is an electronegative radical and R is a protein. This strong thiol binding capacity can cause depletion of cellular glutathione and inactivation of enzymes, thus disregulating biological functions regulated by sulphydryl compounds, including cellular metabolism, maintenance of intracellular redox balances and cell signalling pathways (Guzzi and La Porta 2008; Vas and Monestier 2008).

Acute toxicity is relatively rare and occurs after exposure to inorganic mercury forms, as a consequence of accidental contamination or suicide attempts. Acute exposure to elemental mercury results in lung damage, characterized by chest pain, dyspnea, cough, interstitial pneumonitis and, ultimately, severe impairment of pulmonary function (McFarland and Reigel 1978). Moreover, mercury vapours may acutely induce profound central nervous system effects, including psychotic reactions characterized by delirium, hallucinations and suicidal tendency. Acute ingestion of inorganic ionic mercury (e.g., mercuric chloride) can damage mucous membrane of the mouth, pharynx and intestine. Haematochezia, hypovolemic shock and death can occur in the absence of proper treatment. However, the most serious and frequent systemic effect of inorganic salts of mercury is renal toxicity, characterized by tubular necrosis leading to oliguria and anuria (Zalups 2000). Renal injury does also occur after long-term exposure to inorganic mercury, where glomerular injury predominates, due to direct effects on the glomerular basement membrane and indirect effects mediated by immune complexes.

Prolonged exposure to Hg^0 may induce damage to the nervous system, since this liposoluble form easily crosses the blood–brain barrier, reaches the brain where it is oxidized to mercuric ions, and accumulates as Hg^{2+} in cortex and cerebellum. Symptoms include axonal sensor motor polyneuropathy, hallucinations and mercurial erythrism, a syndrome characterized by excitability, loss of memory, insomnia, extreme shyness and neurocognitive impairments (Vroom and Greer 1972; WHO 1991).

The most common intoxications in humans derive from ingestion of foods, primarily fish, contaminated with methylmercury. Being highly liposoluble, this organic compound is almost completely absorbed through the gastrointestinal tract and can easily cross the blood–brain barrier and the placenta. Levels in the foetal brain are about 5–7 times that in maternal blood (Cernichiari et al. 1995), although epidemiological studies on the developmental neurotoxicology of methylmercury have led to controversial results (see Guzzi and La Porta 2008). In adult, the major toxic effects of methylmercury are on the central nervous system, where loss of neuronal cells may occur in

specific anatomical regions. Signs and symptoms include paresthesia, cerebellar ataxia, dysarthria, constriction of the visual fields and loss of hearing (Bakir et al. 1973; Weiss et al. 2002). The serious health consequences of methylmercury exposure were dramatically manifested in 1953, when an epidemic poisoning caused by consumption of contaminated fish occurred in people leaving around Minamata Bay, in Japan (Tsuda et al. 2009). Apart from many deaths, symptoms due to methylmercury poisoning included mental retardation, cerebral palsy, deafness, blindness and dysarthria, especially in children exposed in utero. However, it is important to underline that the general population does not face significant health risks from methylmercury exposure with the exception of certain groups with high fish consumption.

Exposure to elemental and inorganic forms of mercury can be assessed by measuring mercury levels in either urine or blood, although individuals with a past history of exposure may have elevated levels of the metal in urine but not in blood. The normal upper limit for excretion of mercury in urine is 5 μg/l. By contrast, measurement of mercury in whole blood is the preferred test for exposure to organic mercury, since this form is primarily excreted in the faeces. Mercury in hair may be used to estimate long-term exposure, but potential contamination may make interpretation difficult. Thus, it is important to choose the appropriate test depending on the suspected source of exposure (Baselt 1988; Brodkin et al. 2007).

Poisoning with either inorganic or elemental mercury is routinely treated with dimercaprol (for high-level exposures or symptomatic patients) or penicillamine (for low-level exposures or asymptomatic patients). Although the orally effective chelator 2,3-dimercaptosuccinic acid appears to be an effective chelator for mercury, it has not been approved by the FDA for this purpose (Baum 1999; Risher and Amler 2005).

The short-chain organic mercurials, especially methylmercury, are the most difficult forms of mercury to mobilize from the body, presumably because of their poor reactivity with chelating agents. Since methylmercury compounds undergo extensive enterohepatic recirculation, the use of nonabsorbable mercury-binding substances into the intestinal tract should facilitate their removal from the body. A polythiol resin used for this purpose showed effectiveness in removing the metal and displayed fewer adverse effects than do sulphydryl agents (Bakir et al. 1980; Risher and Amler 2005).

Arsenic

Arsenic is a notoriously poisonous metalloid with many allotropic forms, including a yellow (molecular non-metallic) and several black and grey forms (metalloids). The more toxic form is yellow arsenic (As_4), in which the four atoms are arranged in a tetrahedral structure. This allotrope is the least stable, most reactive, more volatile and less dense than the other forms. It is produced by rapid cooling of arsenic vapour with liquid nitrogen and it is rapidly transformed into the grey allotrope by light.

Arsenic is a widely distributed environmental contaminant that can be found in soil, water and airborne particles as the result of both natural and human activities (Järup 2003; Tchounwou et al. 2003, 2004). The two major industrial processes that lead to arsenic contamination of the environment are smelting of non-ferrous metals and production of energy from fossil fuel, the former being the largest single anthropogenic source of atmospheric pollution (Brooks 2008). Other sources of contamination include the use of arsenic trioxide in the manufacture and use of pesticides and wood preservatives (chromated copper arsenate). Moreover, arsenic is used in the glass manufacturing as arsenic trioxide or as arsenic acid as a bubble dispersant or decoloring agent. High-purity arsenic metal is used for gallium arsenide and indium arsenide semiconductors used in light-emitting diodes and solar cells. Thus, arsenic can be found in discarded electronics, such as computers, televisions, mobile phones, circuit boards, relays and switches (Brooks 2008).

In the past, a number of arsenic compounds have been used as drugs. Among these, arsphenamine was employed to treat syphilis and trypanosomiasis, and arsenic trioxide for various pathological conditions such as psoriasis and, more recently, for cancer (Antman Karen 2001). In 2000, the U.S. Food and Drug Administration approved arsenic trioxide for the treatment of patients with acute promyelocytic leukaemia that is resistant to all-trans retinoic acid (Shen et al. 2001; Miller et al. 2002; Tallman 2007).

Inorganic arsenic is present in groundwater used for drinking in several countries all over the world, as a result of weathering of arsenic-containing minerals exposed by natural processes or disturbed by mining or other anthropogenic activities (Brooks 2008). FDA's standard of quality for bottled water allows no more than 10 μg/l (FDA 2007). Moreover, organic arsenic compounds (such as arsenobetaine) are primarily found in fish, which thus may give rise to human exposure (Järup 2003).

Toxicity of arsenic is strongly related to its chemical form: elemental arsenic, inorganic [e.g., arsenic oxide, As_2O_3; orpiment, As_2S_3; realgar, As_4S_4; arsenic acid, H_3AsO_4; arsine, AsH_3; calcium arsenate, $Ca_3(AsO_4)_2$; lead hydrogen arsenate, $PbHAsO_4$; gallium arsenide, GaAs] and organic compounds [e.g., trimethylarsine, $(CH_3)_3As$; arsenobetaine, $C_5H_{11}AsO_2$]. Inorganic As such as the pentavalent form arsenate (As^{5+}), and the trivalent form arsenite (As^{3+}) are the most aggressive single substance toxicants.

Epidemiological studies have highlighted that arsenite is more toxic than arsenate with regard to cancer risk (Lerman et al. 1983; Bertolero et al. 1987). This may be due to a better cellular uptake and accumulation of the trivalent form, when compared to the pentavalent form.

Nowadays, acute intoxication rarely occurs in western Europe countries, being usually the result of intentional (suicide or homicide) or accidental poisoning, or caused by occupational exposure to arsine gas (Vahidnia et al. 2007). Acute toxicity of arsenic is related to its chemical form and oxidation state. In general, acute toxicity of trivalent arsenic is greater than pentavalent form and, although methylation has been considered a detoxification reaction for many years, more recently several studies have demonstrated that some organic forms may be even more toxic than arsenite (Styblo et al. 2000; Petrick et al. 2001). Symptoms of severe acute arsenic toxicity include gastrointestinal discomfort, vomiting, diarrhoea, bloody urine, anuria, shock, convulsions, coma and eventually death (Hughes 2002).

Chronic exposure to arsenic may affect several systems within the body, including the cardiovascular, nervous and endocrine systems (Hughes 2002; Vahidnia et al. 2007; Iavicoli et al. 2009). One of the hallmarks of chronic toxicity in humans from oral exposure to arsenic is skin lesions, which are characterized by hypo- or hyper-pigmentation and hyperkeratosis (Yeh et al. 1968; Cebrián et al. 1983). Blackfoot disease, a vasoocclusive disease that leads to gangrene of the extremities, has also been observed in individuals chronically exposed to arsenic in their drinking water (Tseng 2005). Other effects of chronic exposure include peripheral neuropathy, encephalopathy, hepatomegaly, cirrhosis, altered haeme metabolism, bone marrow depression, diabetes, proximal tubule degeneration, papillary and cortical necrosis. Moreover, prolonged exposure has been linked to cancer of the skin, bladder, liver and lungs (ATSDR 2007). Indeed, inorganic arsenic is classified by the International Agency for Research on Cancer (IARC 1987, 2004) and by the U.S. Environmental Protection Agency (EPA 1999) as a known human carcinogen.

General population exposure to arsenic is mainly via intake of food and drinking water, and absorption mainly takes place in the small intestine. However, a minimal absorption may also occur from skin contact and inhalation of airborne particles (Enterline et al. 1987; Hertz-Picciotto and Smith 1993; Centeno et al. 2002). Hydrosoluble arsenite and arsenate compounds are more easily absorbed than the less soluble oxides. After absorption, arsenic accumulates mainly in the liver, but also in kidney, heart, lung and, to a lesser extent, in muscle and neural tissue. Given its high affinity for sulphydryl groups, arsenic strongly interacts with keratin of hair and nails; whereas, its chemical similarity with phosphorus facilitates its deposition in bone and teeth, where it is retained for long periods. Moreover, arsenic readily crosses the placenta, and foetal damage has been reported.

Inorganic arsenic is metabolized to organic compounds, mainly represented by methylated metabolites that are rapidly excreted through the urine. Bioavailability and toxicity of arsenic strongly depends on its chemical forms and oxidation state, thus, chemical speciation analysis in biological samples (e.g., urine, fingernails and hair) is crucial to evaluate exposure and potential health risks (Styblo et al. 2000; Mandal et al. 2004).

Methylated arsenic metabolites are produced in the liver by conjugation reactions catalysed by methyltransferases that use S-adenosylmethionine as methyl donor (Aposhian and Aposhian 2006; Cohen et al. 2006). These arsenic metabolites include pentavalent or trivalent monomethylated (MMA) and dimethylated (DMA) compounds. Since methylated species are excreted much faster than inorganic forms, methylation is considered a part of the detoxification program. However, organic arsenic compounds may have deleterious effects on different human cell types, with the methylated trivalent arsenicals significantly more toxic than their pentavalent counterparts (Petrick et al. 2000; Styblo et al. 2000; Thomas et al. 2001). This is of interest since, in the liver, methylation is followed by reduction of pentavalent arsenate to trivalent arsenite, via a reaction that involves glutathione and other thiols (Buchet and Lauwerys 1988).

Arsenic metabolites exert their toxicity by inactivating many enzymes, especially those involved in the production of energy by the cell and in DNA synthesis and repair. In particular, inorganic and organic compounds of trivalent arsenic may interact with thiol groups of proteins and enzymes in their reduced state, thus inhibiting their function (Aposhian et al. 2004; Vahidnia et al. 2007). By this mechanism, arsenic may disrupt several processes implicated in cell metabolism. Inhibition of pyruvate dehydrogenase (PDH), the enzyme crucially involved in Acetyl-CoA production, leads to disruption of the energetic cellular homeostasis (Aposhian and Aposhian 2006) and to the release of apoptosis-inducing factor (AIF) resulting in cell damage and death (Akay et al. 2004). PDH is particularly sensitive to trivalent arsenicals because of their interaction with two sulphydril groups of lipoic acid to form a stable six-membered ring.

Pentavalent arsenic is substituted for phosphorus in many biochemical reactions. In fact, given their chemical similarity, AsO_4^{3-} may replace PO_4^{3-} in a number of biological compounds, including ATP. Being arsenate anion less stable than phosphate, a rapid hydrolysis of high-energy bonds in compound such as ATP may occur. At the level of the citric acid cycle, arsenic inhibits succinate dehydrogenase and, by competing with phosphate, it

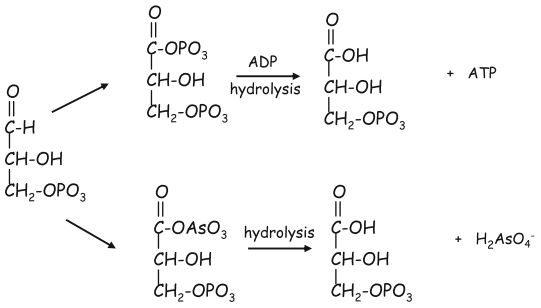

Fig. 6 Arsenolysis

uncouples oxidative phosphorylation, thus inhibiting reduction of NAD, mitochondrial respiration and ATP production. This causes loss of high-energy phosphate bonds and effectively uncouples oxidative phosphorylation, by a mechanism termed arsenolysis (Saha et al. 1999; Hughes 2002) (Fig. 6).

Several mechanisms have been proposed to explain arsenic carcinogenicity (Salnikow and Zhitkovich 2008). Inorganic arsenic has been shown to induce chromosomal aberrations in a number of cell types, and arsenite is more potent than arsenate in producing this effect. Moreover, both inorganic and organic arsenicals are genotoxic either in vitro or in vivo (Hughes 2002). Inhibition of enzymes implicated in DNA repair, such as poly-(ADP-ribose)polymerase, has also been suggested to contribute to the carcinogenic effects of arsenic compounds (Yager and Wiencke 1997). Other mechanisms include alteration of DNA methylation, oxidative stress, increased cell proliferation, co-carcinogenesis and tumour promotion (Hughes 2002).

The concentration of arsenic (and/or its metabolites) in blood, urine, hair or nails is considered a biomarker of exposure (Mandal et al. 2004; Vahidnia et al. 2007). Assessment of arsenic content in hair and nails can be a useful indicator of past arsenic exposure, if care is taken to avoid exogenous contamination. By contrast, speciation of metabolites in the urine is generally the best estimate of recent exposure. Since consumption of certain seafood may confound estimation of inorganic arsenic exposure, it should be avoided before urine sampling.

For the treatment of acute arsenic poisoning, in addition to routine measures aimed at preventing further absorption of the poison (e.g., gastric lavage, activated charcoal), the primary concern is to correct dehydration in order to avoid fatal hypovolemic shock. Dimercaprol and D-penicillamine have been used with successful results, although neurological complications, occurring as late effects of acute poisoning, are often non-responsive to chelation (Vahidnia et al. 2007). After long-term exposure to arsenic, treatment with dimercaprol and D-penicillamine may also be used, although administration of oral penicillamine alone is usually sufficient.

Chromium

Chromium is the 21st most abundant element in the Earth crust, with an average concentration of 100 ppm (Emsley 2001). Several compounds of this transition metal are found in the environment, due to erosion of chromium-containing rocks and volcanic eruptions, and may be found in soil, sea water, rivers and lakes (Kotaś and Stasicka 2000). In most cases, trivalent chromium is the dominating species, although in some geographical areas ground water may contain higher amounts of the hexavalent form (Kotaś and Stasicka 2000).

Chromium and its compounds have a long history of industrial uses in the manufacture of a large number of products. Given the strengthening effect of forming stable metal carbides at the grain boundaries and the strong increase in corrosion resistance, chromium is an important alloying material for steel. Thus, chromium is used in stainless steel (widely used for cookware and cutlery) and in nickel superalloys for jet engines and gas turbines. Other uses include surface coating by electroplating techniques and anodizing of aluminium. Chromate and chromium(III) oxide are widely employed as pigments for metal, glass and synthetic rubies. Moreover, chromium is used in the preservation of wood, in the tanning of leather, for the production of high temperature refractory materials and as catalyst. In addition to occupational exposure of industrial workers, environmental exposure impacts a high number of people drinking chromium-containing water, residing in the vicinity of industrial sites (Zhitkovich 2002; OSHA 2006).

Chromium is probably the most controversial of the transition metal ions in term of its biological activities (Cronin Joseph 2004). Chromium speciation has attracted a great deal of attention, since its chemical species may display differential toxicity. In its most stable oxidation state, Cr^{3+}, it cannot usually cross cell membranes and its toxicity is considered relatively low (De Flora et al. 1990; IARC 1990). Moreover, Cr^{3+} is regarded by many nutritionists as an essential micronutrient for humans, because of its role in glucose and lipid metabolism. Nevertheless, recent data suggest that the potential genotoxic side effects of Cr^{3+} complexes may outweigh their possible benefits as insulin enhancers, and that recommendation for their use as either nutritional supplements or antidiabetic drugs need to be reconsidered (Levina and Lay 2008). In its higher oxidation state (VI), chromium is transported into the cell through anion channels as chromate (CrO_4^{2-}) (De Flora and Wetterhahn 1989), and can be reduced by several intracellular systems (such as glutathione, ascorbate, tocopherols and different enzyme cofactors) to generate stable Cr^{3+} or unstable Cr(IV) and Cr(V) intermediates, all of which are capable of forming complexes with proteins and DNA and generating oxidative stress (Bagchi et al. 2002; Wise et al.

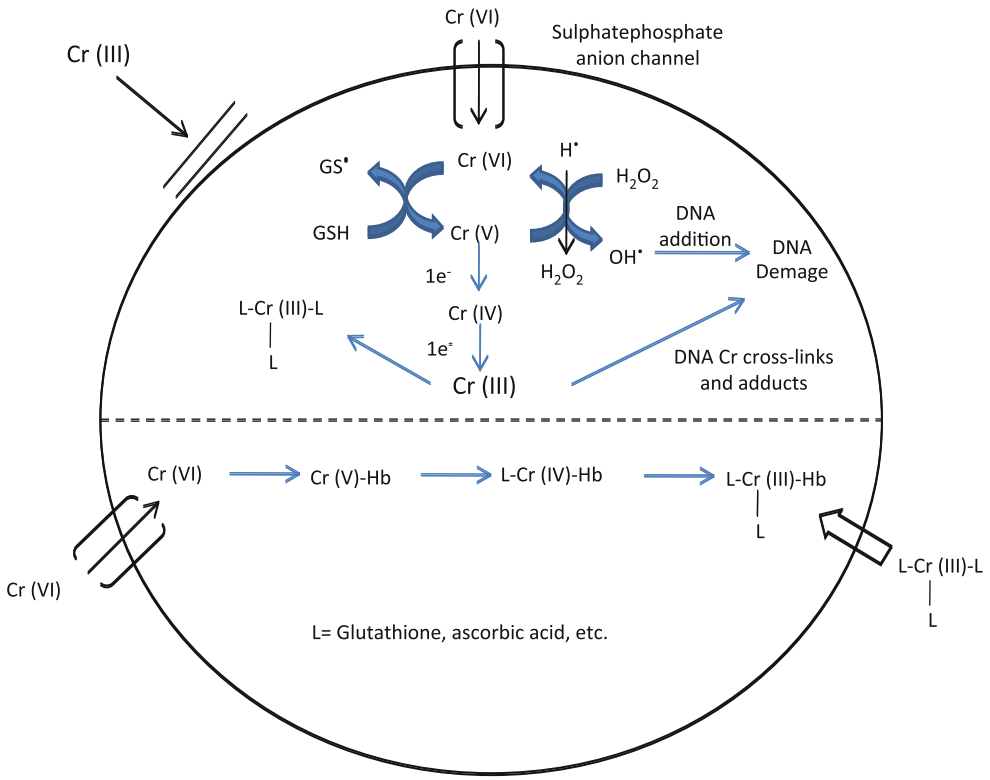

Fig. 7 Pathways involved in Cr metabolism in erythrocytes (bottom) and induction of DNA damage by Cr(VI) (top). Cr(III) is poorly absorbed by cells, while Cr(VI) is brought into the cell via sulphate anion channels. Once in the cell, Cr(VI) is reduced to Cr(III)—a mutagenic form of Cr (adapted from O'Brien et al. 2003)

2002; Levina and Lay 2005; Zhitkovich 2005). Moreover, Cr(VI) induces cytotoxicity and genotoxicity via a variety of mechanisms and is a well-established human carcinogen and a very common occupational hazard (IARC 1990; Levina et al. 2003; Stallings and Vincent 2006) (Fig. 7).

In occupational settings, Cr(VI) exposure mainly occurs through inhalation and may be an important cause of lung cancer (ATSDR 2000). Moreover, chromium compounds may be readily absorbed through the skin and the gastrointestinal tract. This is of interest, since relatively high concentrations of Cr^{3+} may be found in food, such as egg yolk, brewer's yeast, liver, beef, cheese, wine, wholemeal, rye, potatoes and oysters (Kumpulainen 1992; Roussel et al. 2007).

In addition to lung cancer, toxicity due to chromium salts includes hand ulcers, dermatitis, perforation of the nasal septum. Ingestion of hexavalent chromium (chromate or dichromate salts) causes ulceration of the bowel, diarrhoea, and renal and hepatic damage, and there is also evidence of toxicity of hexavalent chromium after cutaneous absorption (see Costa 1997; Katz and Salem 1993).

Evaluation of environmental exposure can be determined by assessing chromium content in urine, plasma, red and white blood cells and, as more recently suggested, in exhaled breath condensate (Coogan et al. 1991; Paustenbach et al. 1997; Goldoni et al. 2006).

Nickel

Nickel is a transition metal, hard and ductile and is one of the five ferromagnetic elements. There is evidence suggesting that it may be an essential trace element for mammals. However, although nickel is present at a concentration of approximately 0.5 nM in the human bloodstream, neither the source of nickel requirement nor a single nickel-dependent enzyme has been detected so far in mammals (Denkhaus and Salnikow 2002; Ragsdale 2009). By contrast, the presence of nickel is essential for eight enzymes (glyoxylase I, aciductone dioxygenase, nickel superoxide dismutase, urease, NiFe hydrogenase, CO dehydrogenase, Acetyl-CoA synthase and methyl-CoM reductase), most of which involve the use and the production of gases that play important roles in the global biological carbon, nitrogen and oxygen cycles (Ragsdale 2009).

In the environment, nickel is primarily found in the form of oxides or sulphides that occur in the earth's crust, or combined with other elements in soils, meteorites and volcanoes. In modern industry, it is used to form alloys with other metals. Approximately 65% of nickel used in western countries is employed to produce stainless steel, and 12% to produce superalloys. Other uses of nickel include the manufacturing of rechargeable batteries, coins, jewellery,

electric guitar strings, catalysts for production of carbon nanoparticles. It is also used for plating and as a green tint in glass.

The toxicity of nickel is dependent on the route of exposure and on the solubility of its compounds. Inhalation is the primary route of human exposure to nickel and may be the cause of acute respiratory symptoms, ranging from mild pulmonary irritation and inflammation to bronchitis, pulmonary fibrosis, asthma and pulmonary oedema (Morgan and Usher 1994). Nickel may be absorbed as the soluble ion Ni^{2+}, while sparingly soluble compounds may be phagocytized. The mucociliary system removes nickel from the respiratory tract resulting in the material entering the gastrointestinal tract. Although gastrointestinal absorption of nickel is poor (1–10%), ingestion of contaminated food and drinking water provides most of the intake of nickel (EPA 1986; Das et al. 2008). The metal is poorly absorbed through the skin, but some compounds such as nickel chloride or sulphate can penetrate occluded skin resulting in up to 77% absorption within 24 h (ATSDR 1988).

Following absorption, nickel is excreted in the urine. In humans most ingested nickel is not absorbed through the gastrointestinal tract and is therefore eliminated in the faeces.

In addition to respiratory symptoms, nickel exposure may cause cardiovascular and kidney disease, as well as allergic dermatitis. However, the most serious concern is represented by its carcinogenetic activity, since Ni^{2+} compounds are classified as human carcinogens (Group 1) and metallic nickel as possibly carcinogenic to humans (Group 2B) by the International Agency for Research on Cancer (IARC 1990), that most likely occurs through non-genotoxic mechanisms induced by Ni^{2+} (Salnikow and Zhitkovich 2008). In general, water-soluble Ni^{2+} compounds display lower toxicity and carcinogenic potential when compared to semisoluble compounds, such as nickel subsulphide Ni_3S_2 (Kasprzak et al. 2003).

The most frequent health effect of nickel in humans is an allergic skin reaction that develops in subjects sensitive to the metal. In fact, nickel is among the most common causes of immediate and delayed hypersensitivity observed in occupationally exposed workers and in the general population. In this regard, nickel is not only an allergen, but also a potential immunomodulatory and immunotoxic agent (Das et al. 2008).

Chelating agents for the treatment of metal intoxications

In order to prevent the deleterious effects of metals, it is crucial to promote their elimination and inactivation. If the therapeutic intervention occurs immediately after the toxicant ingestion, it is possible to limit its absorption by performing a gastric lavage. Alternatively, various human metal intoxications can be efficiently treated by administration of a chelating agent.

Chelators are chemical compounds able to bind the metal with a higher affinity when compared to endogenous ions, and to form a hydrophilic complex that can be easily eliminated. Successful chelation of the toxic metal depends on the nature and properties of the metal and of the chelator (e.g., ionic diameter, ring size and deformability, hardness/softness of electron donors and acceptors), but also on organism-related factors (e.g., route of administration, bioavailability, metabolism, organ and intra/extracellular compartmentalization, excretion) (Andersen and Aaseth 2002).

A good chelating agent should have the following properties:

- capacity of irreversibly bind the toxic metal (chemical affinity for the toxic metal should be higher than the affinity of the metal for the sensitive biological molecules);
- low affinity for essential metals (EDTA, for example, can bind essential metals thus producing toxicity due to their depletion. To overcome this limitation, EDTA is usually administered as a complex with the essential metals for which it displays affinity, e.g. CaNa-EDTA);
- low toxicity of the chelator itself and of the complex it forms with the metal (this is not always achieved, since most chelating agents produce toxic effects. For example, CaNa-EDTA is nephrotoxic, penicillamine causes acute allergy-like reactions, dimercaprol induces tachycardia and nausea);
- effectiveness after oral administration;
- limited metabolic transformation of the chelating agent and of the chelate;
- accessibility of the adduct to urine and bile to allow rapid elimination of the metal;
- stability of the chelator-metal complex at the physiological (and urinary) pH

The main chelating agents used to treat metal intoxications are dimercaprol, ethylene diamine tetraacetic acid (EDTA), dimercaptosuccinic acid (DMSA) and dimercaptopropionic sulphonate (DMPS), D-penicillamine and deferoxamine.

Dimercaprol (2,3-dimercaptopropanol), also known as British anti-Lewisite (BAL), was developed by British biochemists during the Second World War as an antidote for dicholorovinyl arsine (Lewisite) the now-obsolete Arsenic-based chemical warfare agent (Peters et al. 1945).

BAL is a dithiol chelating agent, with high affinity for the "sulphur-seeking" metals, such as mercury and arsenic. BAL forms heterocyclic ring complexes with some heavy metals, preventing or reversing the binding of metallic cations to body ligands, such as the essential sulphydryl-containing enzymes.

Major drawbacks for the clinical use of BAL include the following: its low therapeutic index; its tendency to redistribute arsenic and mercury to brain and testes; the need for (painful) intramuscular injection and its unpleasant odour. Toxic effects of BAL include nausea, vomiting, abdominal pain, high fever, hypertension, tachycardia, thrombocytopaenia and nephrotoxicity (Mückter et al. 1997). Administration of the glucoside derivative (BAL-glucoside) allows intravenous injection of the chelator and results in diminished toxicity due to the increased polarity of the molecule (Danielli et al. 1947).

BAL is used as an antidote for arsenic, mercury and lead (in conjunction with EDTA), but evidence suggests its effectiveness also in the treatment of antimony, bismuth, chromium, mercury, gold and nickel poisoning. It is not indicated for the treatment of iron, cadmium, selenium, silver and uranium poisoning, since the complexes it forms with these elements are more toxic, especially to the kidneys, than the metal alone.

Because it is a lipophilic drug, dimercaprol penetrates rapidly the intracellular space. The highest concentrations are found in the liver, kidneys, brain and small intestine. Biological half-life is short and metabolic degradation and renal excretion are complete within 6–24 h. The dimercaprol–metal complexes dissociate rapidly in the body, especially in an acid internal medium; thus, alkalinization of the urine may prevent this dissociation and protect the kidneys from metal and BAL nephrotoxicity.

The breakthrough in the development of chelation therapy came after the introduction of EDTA, initially used to treat lead intoxication. EDTA is a synthetic amino acid that can form complexes with several metals, such as chromium, iron, mercury, copper, lead, zinc, aluminium, manganese, calcium and magnesium.

Chelation of iron and copper occurs when they are not included in enzymatic complexes or transport systems, namely only when they are in an unbound (free) state, thus providing a very selective mechanism of action. However, the value of EDTA as a clinical chelating agent was reduced by the need for slow intravenous administration, low intestinal uptake, exclusive extracellular action and high stability constants with essential metals (e.g., zinc; Powell et al. 1999). Moreover, the major toxic effect of this chelator occurs on the renal system with necrosis and hydropic degeneration of tubular cells.

EDTA is administered as $CaNa_2EDTA$ by slow endovenous infusion (lasting not less than 3 h). It has a very short half-life (45 min to 1 h) and is eliminated in its metal-complexed form by the kidney (95%) and the liver (5%).

The effectiveness of DMSA and DMPS against arsenic and lead poisoning was demonstrated in the early eighties. When compared to BAL, these newer chelating agents show several advantages, such as a significantly lower toxicity and effectiveness after oral or intravenous administration (Kalia and Flora 2005). DMSA and DMPS are efficient antidotes for intoxications with several divalent metals besides lead and mercury, as well as some organometal or metalloid compounds (Andersen 1999; Aposhian et al. 1995). Adverse reactions during treatment with these chelators include gastrointestinal discomfort, skin reactions, mild neutropaenia and elevated liver enzymes.

Penicillamine (3,3-dimethylcysteine) is used as D-form since L-penicillamine is toxic (it inhibits the action of pyridoxine). D-Pencillamine chelates mercury, lead, copper and iron to form stable and hydrosoluble complexes that are excreted by the urine. This chelator is also used to treat cystinuria, since it binds with cysteine to yield a mixed disulphide which is more soluble than cystine, thus avoiding formation of cystine stones.

D-Penicillamine is absorbed through the gastrointestinal tract and therefore can be administered orally. The improvement of metal intoxication symptoms is usually observed after some weeks of treatment. The major toxic effect of penicillamine is antagonising pyridoxine and inhibiting pyridoxine-dependent enzymes, such as transaminases. Other adverse effects include glomerulonephritis and hypersensitive allergic reactions such as fever, skin rashes, leucopaenia and thrombocytopaenia (Shannon et al. 1988). In the elderly, the risk of haematologic and renal toxicity is increased. Toxicity of D-penicillamine can be potentiated by association with phenylbutazone.

Deferoxamine is used in severe, acute iron poisoning to facilitate the removal of the metal from the body (Henretig et al. 1983; Mann et al. 1989; Cheney et al. 1995). It is usually associated with standard treatment measures such as induction of emesis, gastric lavage, whole bowel irrigation, clinical control of shock and correction of acidosis.

Acute iron poisoning usually follows a biphasic course: within the first 30 min and up to several hours vomiting occurs, followed, 6–12 h later, by abdominal pain, diarrhoea, and eventually lethargy, hyperglycaemia and fever. More severe complications may occur after weeks or months after the acute episode.

Deferoxamine displays high affinity for the ferric form (Fe^{3+}) of the metal that specifically chelates ferritin, hemosiderin and, to a lower degree, transferrin. By contrast, deferoxamine does not seem to chelate iron ions included in the molecules of haemoglobin, myoglobin and cytochromes. Deferoxamine displays low affinity for Fe^{2+} and a very low affinity for Ca^{2+}. The complex formed with Fe^{3+} (ferrioxamine) is very stable and hydrosoluble and is eliminated by renal excretion.

This chelator has to be administered by parenteral route, since gastrointestinal absorption is low.

In acute iron poisoning, the drug is administered by intravenous infusion at doses between 15 and 90 mg/kg

every 8 h. Administration by intramuscular injections (90 mg up to 1 g/kg) can also be performed, including subcutaneous infusions (20–40 mg/kg for 8–24 h) for chronic intoxications. Whichever route is chosen, the daily dose should not exceed 6 g. After treatment, the patient should be monitored to prevent shock (24 h later) and gastrointestinal complications (2 weeks later).

Deferoxamine can also be used to treat patients exposed to aluminium intoxications, including those maintained with haemodialysis. The main side effects include urticaria, skin rash, hypotension, respiratory distress syndrome, auditory and ocular toxicity.

Conclusions

Metal intoxications represent a real health problem in most industrialized countries. The chemical properties of metals and of their compounds strongly affect their toxico-kinetic (e.g., absorption, distribution and excretion) and toxico-dynamic properties. Despite numerous studies have attempted to elucidate the mechanisms implicated in their toxicity, further studies are still needed in order to improve pharmacological treatment. Indeed, chelating agents are the only drugs nowadays available to limit metal toxicity, and their use is often limited by their lack of selectivity, making it urgent to identify novel chemical compounds that allow to specifically remove the toxic metal from the body without affecting physiological ionic homeostasis.

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