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Effects of the metals on dihydropteridine reductase activity

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Abstract

Metals are the oldest toxins known to human. Particularly, occupational and environmental exposure to aluminium, lead, mercury, cadmium, and manganese cause serious health problems by interaction with biological systems. Cellular targets of these metals are mostly specific biochemical processes (enzymes) and/or membranes of cells and organelles. To prevent and/or reduce the untoward or irreversible toxic effects of the metals by using biomarkers are as important as to know and to understand of their toxicity mechanisms. Dihydropteridine reductase (DHPR), which possessed essential thiol groups at the activite site, plays a crucial role in the maintenance of tetrahydrobiopterin (BH_4). BH_4 is the cofactor in the synthesis and regulation of neurotransmitters. A limited number of the evidences have shown that DHPR may be a target for the metals. Therefore, the present study was designed to assess possible in vitro effects of the commonly exposed metals on the enzyme activity. It was found that aluminium, cadmium, mercury, di-phenyl mercury, lead, diethyl lead, in chloride forms, and manganese, in sulphate form, led to statistically significant decreases in DHPR activity, in a concentration-dependent manner, in vitro.

Keywords: Dihydropteridine reductase; Metal; Biomarker; Environmental pollutants

1. Introduction

Tetrahydrobiopterin (BH_4) is the cofactor in the synthesis, and regulation of neurotransmitters, mammalian aromatic amino acid monooxygenases, oxidative cleavage of ether lipids and nitric oxide production. Aromatic amino acid monooxygenases regulate the biosynthesis of neurotransmitters, dopamine, noradrenaline, epinephrine and serotonin by hydroxylating phenylalanine, tyrosine and tryptophan. As shown in Fig. 1, BH_4 is converted to the quinonoid dihydrobiopterin, while BH_4 is mainly regenerated by dihydropteridine reductase (DHPR) enzyme (Kaufman, 1993; Nichol et al., 1985; Whitely et al., 1993). A block in the BH_4 pathway leads to the accumulation of phenylalanine and the altered production of neurotransmitters. This situation may cause severe neurological illness (Jeeps et al., 1986; Altmann et al., 1989; Altindag and Sahin, 1996). It has been recognised that many diseases, some drugs, and xenobiotics, including metals such as aluminium (Altmann et al., 1987, 1989; Bolla et al., 1991), lead (Blair et al., 1982), gallium and scandium (Cutler and Blair, 1987) may cause changes in BH_4 concentration and/or DHPR enzyme activity.

Metals differ from other toxic substances as they are neither be created nor be destroyed by human. Nevertheless, their utilization influences the potential effects on health at least in two major ways: first, by environmental transport, that is, by human or anthropogenic contributions, to the air, water, soil, and food, and second, by altering the specification or biochemical form of the element. Knowledge of the effects of metals on critical or specific organs provides insight into which metal may be responsible for a specific effect. The toxic effects of the metals usually originated from an interaction between the free metal ions and the toxicological target. Blood and/or urine are the most accessible biological

Abbreviations: ATP, adenosine 5′-triphosphate; $AlCl_3$, aluminium chloride; $CdCl_2$, cadmium chloride; DHPR, dihydropteridine reductase; $PbCl_2$, lead chloride; $MnSO_4.H_2O$, manganese sulphate monohydrate; $HgCl_2$, mercury chloride; NADH, nicotinamide adenine dinucleotide, reduced form; KCl, potassium chloride; KOH, potassium hydroxide; SPSS, Statistical Package for the Social Sciences; BH_4, tetrahydrobiopterin; Tris-HCl, Tris-hydrochloric acid.

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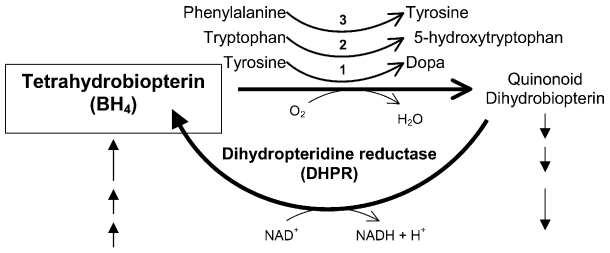

Fig. 1. The critical role of dihydropteridine reductase in neurotransmitter metabolism: 1, tyrosine hydroxylase; 2, tryptophan hydroxylase; 3, phenylalanine hydroxylase.

samples for measuring whether there is an exposure or dose, while they are sometimes referred to as indicator samples (Goyer and Clarkson, 2001).

Among the toxic metals, aluminium is known to interact with a number of proteins and with cofactors that are involved in intermediary metabolism. Aluminium combines with adenosine 5′-triphosphate (ATP) to form ATP-aluminium, a competitive inhibitor of hexokinase and inhibits catechol-*O*-methyltransferase, ceruloplasmin, cholinesterase, colin acetyl-transferase, glycerokinase, magnesium-ATPase, calmodulin, adenylate cyclase, and *d*-aminolevulinic acid dehydratase. Aluminium is ubiquitous in the environment, comprising 8% of the earth's crust, making it the third most abundant element (Nayak, 2002). Lead is also a widely distributed toxic metal and is practically detectable in all phases of the inert environment and in all biological systems. Mechanisms of toxicity include damage to the membranes, impairment of the energy metabolism, and direct interference with the neurotransmitters and haem syntheses. Mercury is a commonly found substance in the environment. It is mostly used in the industry for chlor-alkali production and in the manufacture of electrical apparatus. Mercurials are attracted to sulfhydryl groups in the body and are bound to proteins, onto membranes, and into enzymes, altering their normal functions. Cadmium is widely distributed in the environment at relatively low concentrations. Cadmium poisoning is a rare type of heavy metal poisoning associated with industrial exposure, ingestion of contaminated shellfish, or drinking acidic beverages from contaminated vessels. Manganese is an essential element and also a cofactor for a number of enzymatic reactions particularly those involved in phosphorylation, cholesterol, and fatty acid syntheses. It should be considered that chronic exposure to manganese dust can cause toxic effects in the lungs and the central nervous system (Goyer and Clarkson, 2001; Seiler and Sigel, 1988).

The main goal of the present study was to evaluate whether there were any effects of aluminium, cadmium, manganese, lead, diethyl lead, mercury, and di-phenyl mercury on DHPR activity in order to contribute to the explanation of their toxicity mechanisms.

2. Materials and methods

2.1. Chemicals

All of the chemicals used in the study were of analytical grade and were purchased from Sigma Co. (St. Louis, MO, USA) and Merck Co. (Darmstadt, Germany). Aluminium chloride ($AlCl_3$), cadmium chloride ($CdCl_2$), manganese sulphate monohydrate ($MnSO_4.H_2O$), lead chloride ($PbCl_2$), mercury chloride ($HgCl_2$), diethyl lead and di-phenyl mercury, in chloride forms, were used to prepare the metal solutions.

2.2. Preparation of the enzyme extract

Blood dropped on a filter paper was used in the experiment. In order to prevent personal variations, all of the experiments were performed with the blood of the same, healthy, and non-smoker person who works as a staff in the department and did not receive any medication during this period.

The dried blood spots were cut into discs having 5 mm diameter and incubated for 3 h at +4 °C with the solutions in 150 mM potassium chloride (KCl) of 1–1000 μM $AlCl_3$ or 1–100 μM $HgCl_2$ or 0.1–5 μM di-phenyl mercury chloride or 1–100 μM $CdCl_2$ or 1–100 μM $PbCl_2$ or 5–50 μM diethyl lead chloride or 0.1–100 μM $MnSO_4.H_2O$. Each enzyme extract was used for the measurement of the DHPR activity.

Under the same assay conditions, the enzyme extract as a control was prepared by using 150 mM cold KCl solution without any metal.

2.3. Measurement of DHPR activity

Each enzyme extract was added to the reaction medium that contains 51 mM Tris–hydrochloric acid (Tris–HCl), pH 7.6, 1 mM ferricytochrome C in 5 mM Tris–HCl containing 0.1 M KCl, pH 7.4, 0.5 mM nicotinamide adenine dinucleotide, reduced form (NADH) in 0.01 M potassium hydroxide (KOH), 0.25 mM 6-methyl-tetrahydropterine in 0.01 M HCl in a final volume of 2 ml.

The changes in enzyme activity were measured at 550 nm at room temperature with a spectrophotometric method based on reduction of ferricytochrome C in presence of NADH. DHPR activity was expressed as nanomoles of cytochrome C reduced per minute relative to the 5 mm diameter of blood spots (Arai et al., 1982).

The alteration in the activity was expressed as a percentage of control activity. All measurements were done in duplicate.

2.4. Statistical analysis

Data processing was carried out using a Statistical Package for the Social Sciences (SPSS) for Windows

packed program. Spearman-rank correlation was used to examine the interrelation of DHPR activity with the various metal concentrations. The P values <0.05 were considered significant.

3. Results

Incubation with aluminium resulted in a decrease of DHPR activity in a dose-related manner (Fig. 2a). The enzyme activity was negatively correlated with aluminium concentrations ($Rs=-0.900$; $P<0.05$). DHPR activity was reduced when the blood spots were incubated with mercury or di-phenyl mercury (both $Rs=-1.000$; $P<0.05$). The effects of inorganic and organic mercury on the enzyme activity are indicated in Fig. 2b and c, respectively. A similar decline in the activity of the enzyme by cadmium was observed ($Rs=-1.000$; $P<0.05$). Fig. 2d shows the changes in DHPR activity depending on the concentration of cadmium. The effects of inorganic and organic lead on DHPR activity are shown in Fig. 2e and f, respectively. There was a negative correlation between lead chloride concentration and the activity of the enzyme ($Rs=-1.000$; $P<0.05$). The inverse relationship was also found for diethyl lead and the enzyme activity

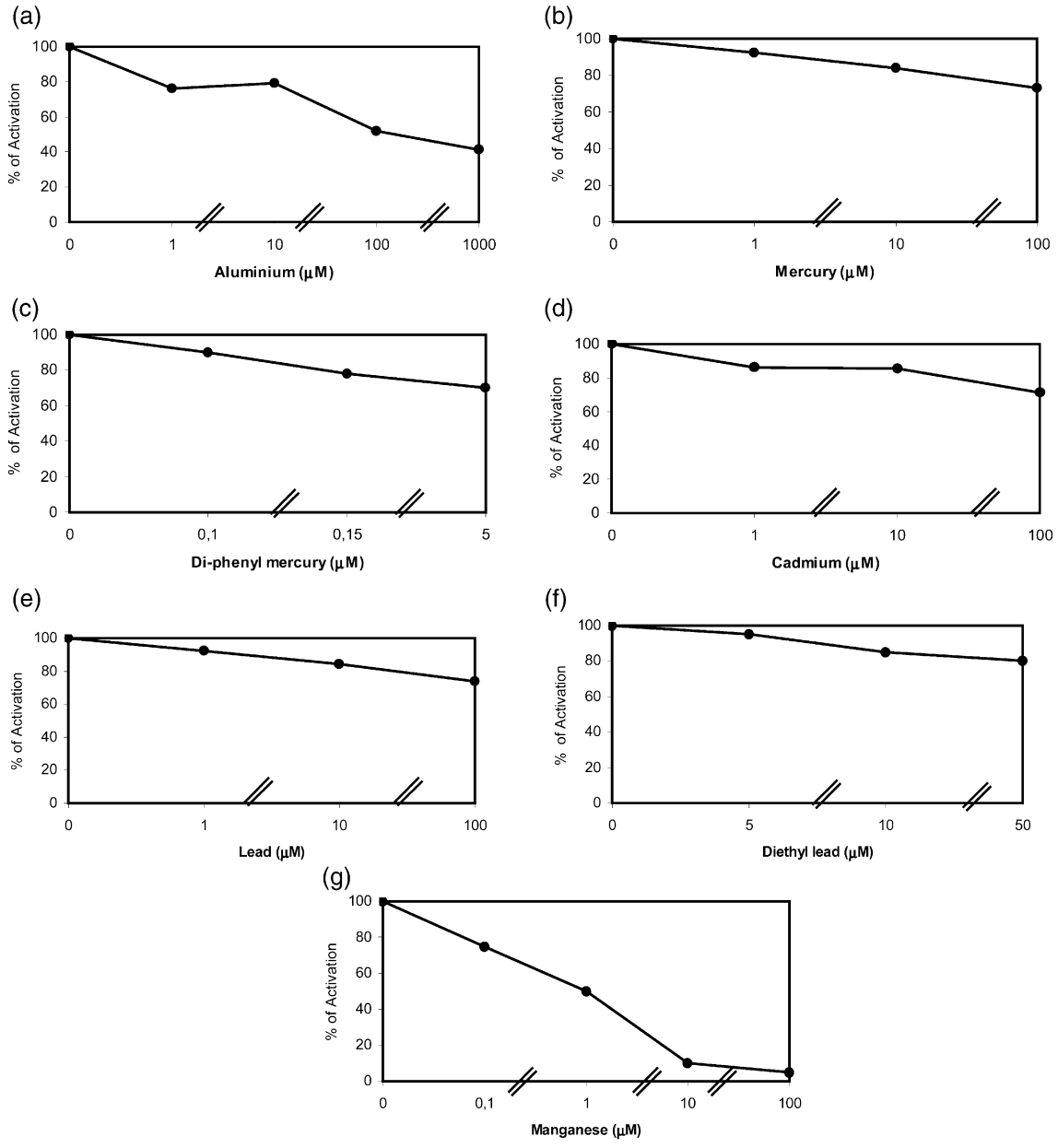

Fig. 2. In vitro effects of the metals on dihydropteridine reductase activity.

($Rs = -1.000$; $P < 0.05$). As indicated in Fig. 2g, the inhibition by manganese was depended on its concentration ($Rs = -1.000$; $P < 0.05$).

4. Discussion

It is known that biochemical enzymes are main cellular targets in metal toxicity. Therefore following the establishment of the optimum conditions for the measurement of the enzyme activity, in vitro effects of various concentrations of aluminium, mercury and diphenyl mercury, cadmium, lead and diethyl lead, and manganese on the DHPR activity were investigated. When blood dropped papers incubated with aluminium at 1–1000 μM and with manganese at 1–100 μM concentrations, changes in the enzyme activity were from 76 to 41% and 75 to 5%, respectively. 50% enzyme inhibition was shown at both 100 μM aluminium and 1 μM manganese. On the other hand, incubation with 1–100 μM cadmium resulted in a decrease of DHPR activity, as 86–71%. Toxicity is determined by dose at the cellular level, and such factors as chemical form or species and ligand binding become critical. Alkyl compounds, in comparison to inorganic forms are more lipid-soluble and pass readily across biological membranes unaltered by their surrounding medium. They are only slowly dealkylated or transformed to inorganic salts. Hence, their excretion tends to be slower than inorganic forms, and the pattern of toxicity of organic forms tends to differ from inorganic forms (Goyer and Clarkson, 2001). As our results have also shown that alkyl compounds of lead and mercury were more effective for inhibition of the enzyme than their inorganic compounds. Inorganic salts of mercury, and lead led to decreases in the enzyme activity at 1–100 μM concentrations (92–17% and 92–74%, respectively). The organic forms of mercury and lead, di-phenyl mercury and diethyl lead, showed similar trends, but in much lower concentrations, 0.1–5 μM (90–70%) and 5–50 μM (95–80%), respectively. According to these preliminary results, it seems to be difficult to make precisely conclusions at the moment. However the inhibitory effects of the metals on the enzyme activity may be expressed in the decreasing order of effectiveness is as follows: diphenyl mercury, manganese, mercury, diethyl lead, cadmium, lead, aluminium. It has been showed that aluminium, lead, scandium and gallium reduced the blood DHPR activity in humans and animals (Altmann et al., 1987, 1989; Blair et al., 1982; Bolla et al., 1991; Cutler and Blair, 1987). The similar results were observed by platinum in human brain (Armarego and Ohnishi, 1987).

Our preliminary in vitro results may also explain the underlying mechanisms of neurotoxicity of these metals. Since DHPR is important for the regeneration of BH_4, a required cofactor in hydroxylation reactions of biogenic amines (Abelson et al., 1979; Kaufman, 1993; Nichol et al., 1985; Whitely et al., 1993), impairment of the reduced pteridine cofactor may trigger neurological and mental deterioration due to impaired catecholamine and indoleamine neurotransmitter and hormone biosyntheses (Webber et al., 1988). The tested metals have been shown to be the inhibitors of DHPR to some extent. The inhibition of this essential functional site of the activity by these metals is possible, although the in vivo findings have not been supported these in vitro results, yet. On the other hand, it is recognised and well documented that, the sulfhydryl-reactive metals such as mercury, cadmium, lead and arsenic are particularly insidious and can affect a vast array of the biochemical processes, but affinities of all the metals to thiol groups are not at the same degree.

In conclusion, further but in vivo experiments are apparently needed to confirm and to help to clarify these in vitro results. The results may be helpful to understand and explain the possible key role of the enzyme in toxicity mechanisms of these metals. Moreover, the screening of the dihydropteridine reductase enzyme activity may be a useful and early biomarker for evaluation of occupational or environmental exposure to the metals.

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