Experimental and Toxicologic Pathology 68 (2016) 365–370

Contents lists available at ScienceDirect

Experimental and Toxicologic Pathology

journal homepage: www.elsevier.de/etp

Short communication

Effect of cadmium administration in hyperhomocysteinemic mice due to cystathionine beta synthase deficiency

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ARTICLE INFO

Article history:
Received 22 March 2016
Accepted 2 May 2016

Keywords:
Hyperhomocysteinemia
Mice
Liver
Cadmium
Xenobiotic metabolizing enzymes
Docking

ABSTRACT

Homocysteine, a sulfur-containing amino acid formed during the metabolism of methionine, is commonly slightly elevated in the plasma of the general population. Additionally, we previously found that cystathionine beta synthase-deficient mice, a murine model of hyperhomocysteinemia, exhibit altered activities of xenobiotic metabolizing enzymes (XME), which dispose of foreign chemicals, in the liver. Thus, hyperhomocysteinemia may result in susceptibility to xenobiotics like cadmium, a heavy-metal toxicant found in drinking water, atmospheric air, and food. Consequently, we exposed hyperhomocysteinemic mice to cadmium via their drinking water for one month to analyze the combined effects of hyperhomocysteinemia and cadmium exposure in liver. No difference in plasma homocysteine level was found after cadmium administration in control and hyperhomocysteinemic mice, but the glutathione level was significantly lower in exposed hyperhomocysteinemic mice compared to control mice, reflecting oxidative stress. We therefore analyzed the effect of Cd administration on hepatic XMEs known to be dysregulated in hyperhomocysteinemic mice: paraoxonase 1, a phase I XME, and NAD(P)H:quinone oxidoreductase, a phase II XME. Cadmium exposure negatively affected activity of paraoxonase 1, a calcium-dependent enzyme. Thus, we analyzed another calcium-dependent enzyme known to be dysregulated in liver of hyperhomocysteinemic mice, calpain, which was also significantly lower after cadmium administration. A comparison of the calculated affinities of cadmium docking versus calcium redocking suggested that cadmium ions may inhibit enzymatic activities by preventing the binding of calcium ions. Moreover, the increased NAD(P)H:quinone oxidoreductase activity observed after cadmium administration could indicate the presence of protective mechanisms in liver of mice. In conclusion, although cadmium administration had no effect on plasma homocysteine level, its effects on plasma glutathionine level suggest a susceptibility to cadmium in the condition of hyperhomocysteinemia, which could be countered by an increased NAD(P)H:quinone oxidoreductase activity.

1. Introduction

Homocysteine (Hcy), a sulfur-containing amino acid formed during the metabolism of methionine, is commonly slightly elevated in the plasma of the general population. Hcy can be converted in glutathione (GSH), a powerful antioxidant and detoxifying agent. Further, an elevated Hcy level is observed in about 30% of patients with cerebral, coronary, and peripheral vessel disease (Clarke et al., 1991; Verhoef et al., 1997). Several factors affect Hcy levels; for example, cigarette smoke and coffee

Abbreviations: CBS, cystathionine beta synthase; GSH, glutathione; Hcy, homocysteine; HHcy, hyperhomocysteinemia; HO-1, heme oxygenase I; PON1, paraoxonase 1; NQO1, NAD(P)H:quinone oxidoreductase; XME, xenobiotic-metabolizing enzyme.

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http://dx.doi.org/10.1016/j.etp.2016.05.001
0940-2993/

are two environmental factors associated with increased plasma Hcy concentrations (Refsum et al., 2006).

Indeed, elevated plasma Hcy levels have been associated with impaired liver function, emphasizing the liver's central role in metabolism of Hcy (Finkelstein 1998; Stead et al., 2000). We previously found that activity of hepatic paraoxonase 1 (PON1), a Ca^{2+}-dependent-phase I xenobiotic-metabolizing enzyme (XME) synthesized by the liver with anti-oxidative properties within the circulating system, is downregulated in cystathionine beta synthase (CBS)-deficient mice, a murine model of hyperhomocysteinemia (HHcy) (Robert et al., 2003). HHcy also results in reduced hepatic activity of NAD(P)H:quinone oxidoreductase (NQO1), a phase II XME (Noll et al., 2011). XMEs function in the liver to metabolize foreign chemicals, and the regulation of phase I and phase II XMEs can be altered by pro-oxidant environmental pollutants (Maier et al., 2000; Garg et al., 2008). The altered activities of these XMEs under conditions of HHcy could thereby result in susceptibility to pollutants. For example, cigarettes and coffee, which are associated with increased Hcy levels, also contain cadmium (Cd), an occupational and environmental toxicant that is found in drinking water, atmospheric air, and foods (Moulis and Thévenod, 2010). Thus, smoking cigarettes or consuming coffee could promote increased Hcy levels, thereby increasing an individual's susceptibility to toxicity from the Cd found in these sources.

Cd has a long half-life (20–30 years) in the human body (Flora et al., 2008) and accumulates in tissues, especially the liver and kidney (Nakamura et al., 2012). Indeed, Cd is recognized to provoke liver damage (Gebhardt, 2009). Interestingly, Cd is reported to interfere with cellular Ca^{2+} homeostasis, and the Ca^{2+}-dependent cysteine proteases, calpains, have been demonstrated to be dysregulated in liver of HHcy mice (Hamelet et al., 2009a,b). Thus, we hypothesized that altered xenobiotic metabolism under conditions of HHcy and Cd exposure would lead to altered enzyme activity and cellular homeostasis in the liver. Here, we exposed CBS-deficient mice, a model of HHcy, and control mice to chronic but relatively low and environmentally relevant doses of Cd to identify the combined effects of HHcy and Cd exposure on plasma Hcy level and on liver XME activity known to be dysregulated in HHcy.

2. Materials and methods

2.1. Mice and genotype determination

All animal care was conducted in accordance with internal guidelines of the French Agriculture Ministry for animal handling. Mice were housed in a controlled environment with unlimited access to food and water on 12-h light/dark cycle. Number of mice and suffering were minimized as possible. Mice heterozygous for targeted disruption of the Cbs gene (Cbs +/−) were generously donated by Dr. N. Maeda (Department of Pathology, University of North Carolina, Chaped Hill, NC, USA) (Watanabe et al., 1995). *Cbs* +/− mice, on a C57BL/6 background were obtained by mating male *Cbs* +/− mice with female wild-type C57BL/6 (*Cbs* +/+) mice. DNA isolated from 4 week-aged mice tail biopsies was subjected to genotyping of the targeted CBS allele using a polymerase chain reaction (PCR) assay (Watanabe et al., 1995). Female from each genotype from the same litter (two months of age) were used.

2.2. Cd administration

Cbs +/+ and Cbs +/− mice were randomly divided into 2 groups and maintained for one month on the following diets before the experiments: control diet (−) with water without Cd chloride (Sigma-Aldrich), and control diet with low Cd concentration (5 mg/kg/d of Cd chloride) in the drinking water (Thijssen et al., 2007).

2.3. Preparation of plasma samples, tissue collection, and plasma assays

Upon euthanization of mice, blood samples were obtained by retro-orbital sinus sampling with heparinized capillaries, collected into tubes containing a 1/10 volume of 3.8% sodium citrate, and immediately placed on ice. Plasma was isolated by centrifugation at 2500*g* for 15 min at 4 °C. Livers were harvested, snap-frozen, and stored at −80 °C until use. Plasma total Hcy, defined as the total concentration of Hcy after quantitative reductive cleavage of all disulfide bonds, and total glutathione (GSH) were assayed using the fluorimetric high-performance liquid chromatography (HPLC) method as previously described (Ducros et al., 2002).

2.4. Enzyme activities

PON1 activity assay was performed on 100 μg of liver protein extracts. PON1 arylesterase activity toward phenyl acetate was quantified spectrophotometrically using 20 mM Tris-HCl (pH 8.3), 1 mM CaCl2, and 10 mM phenyl acetate. The reaction was performed at room temperature by measuring the appearance of phenol at 270 nm every 10 s for 1 min using a spectrophotometer (Lambda XLS, PerkinElmer). NQO1 activity was assayed on 150 μg of protein extracts as described (Benson et al., 1980). Proteins were incubated for 8 min at room temperature in PBS containing 0.07% bovine serum albumin (pH 7.4) and 0.01% Tween-20. Then, a mixture containing 0.2 mM β-nicotinamide adenine dinucleotide, reduced (NADH), 5 μM flavin adenine dinucleotide (FAD), and 25 mM Tris-HCl (pH 7.4) was added to the protein preparations. Two conditions were prepared, with or without 10 μmol of dicoumarol (Calbiochem, MERCK) used to specifically block NQO1. The reaction was started by adding 40 μM of 2,6-dichlorophenolindophenol (DCPIP). The reduction of DCPIP was assayed by measuring the absorbance at 600 nm every 30 s for 3 min using a spectrophotometer (Lambda XLS, PerkinElmer). NQO1 activity was determined by subtraction of the activity recorded in the presence of dicoumarol. Calpain activity was measured using the fluorogenic peptide *N*-succinyl-Leu-Tyr-7-amido-4-methylcoumarin as described (Hamelet et al., 2009a), with 40 μg of liver extract.

2.5. Molecular docking

The molecular docking studies were performed using AutoDock 4 program (Morris et al., 2009). The structure of human PON1, PDB ID: 1V04 (Harel et al., 2004), and human calpain, PDB ID: 1ZCM (Li et al., 2006), were downloaded from the Protein Data Bank (Berman et al., 2000). All the atoms that do not belong to the protein have been removed. All hydrogen atoms were added to the proteins structures using PROPKA program (Sondergaard et al., 2011; Olsson et al., 2001) through the PDB2PQR server (Dolinsky et al., 2004). Gasteiger charges were added to the proteins structures and the non-polar hydrogens were merged onto their respective heavy atoms using AutoDockTools program. Cd parameters (radius: 1.30 Å; well depth: 0.55 kcal/mol; and charges: +1.0 e) were set by both computations and homology with other ion parameters available in AutoDock force field as it is recommended by the developers of AutoDock. For each proteins, blind docking experiments, i.e., on the whole surface, were performed to identify the most favorable interaction sites. A grid of $126 \times 126 \times 126$ points with a spacing of 0.56 Å, in which the docking computations were carried out, was centered on the mass center of the studied protein. To identify the best protocol for the Cd docking, we have tested the ability of several research