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Neurohepatic toxicity of subacute manganese chloride exposure and potential chemoprotective effects of lycopene

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ABSTRACT

Excess manganese (Mn) is potentially toxic resulting in a permanent neurodegenerative disorder, clinically known as "manganism" that is distinctive for hepaticencephalopathy. The present study was designed to explore the toxic impacts of subacute Mn exposure on brain and liver tissues, and the relative abilities of lycopene in averting such neurohepatic damage. Rats were daily injected with $MnCl_2$ (0 or 6 mg/kg, i.p.) 20 days after lycopene administration (0 or 10 mg/kg, p.o.), and killed 4 weeks after $MnCl_2$ exposure. $MnCl_2$-induced lipid peroxidation and perturbation in antioxidant system, increase of acetylcholinesterase, aminotransferases, and decrease alkaline phosphatase, and lactate dehydrogenase activities with hyperglycemia as demonstrated by Alzheimer type II astrocytosis, and periportal hepatic necrosis and apoptosis were prevented by lycopene. However, lycopene did not prevent the increased body burden of Mn and the altered Fe and Cu homeostasis induced by $MnCl_2$. Glutathione *S*-transferase and catalase activities, and glutathione content were reduced in $MnCl_2$-challenged rats, and sustained by lycopene. Our results indicate that although lycopene failed to reduce Mn concentration or retain disturbed elemental status; it appears to be a highly effective in alleviating its neurohepatic deleterious effects by preventing lipid peroxidation, hyperglycemia and changes in the activity of acetylcholinesterase and hepatobiliary enzymes, and antioxidant pathways.

1. Introduction

Manganese (Mn) is a naturally occurring trace element commonly found in the environment; it is both nutritionally essential and potentially toxic (Erikson et al., 2005). It plays an important role in a number of physiological processes by serving as a constituent of some enzymes and an activator of others involved in the regulation of amino acid, protein, lipid, and carbohydrate metabolism (Zheng et al., 2011). Mn is an important cofactor for a variety of enzymes in the brain, including the anti-oxidant enzymes Mn-superoxide dismutase and glutathione synthetase (Taylor et al., 2006), as well as those involved in neurotransmitter synthesis and metabolism (Golub et al., 2005). However, elevated brain Mn levels associated with chronic exposure to excessive amounts of Mn in occupational settings (Montes et al., 2008) or secondary to liver diseases, prolonged parenteral nutrition therapy (Dobson et al., 2004), or iron deficiency (Kim et al., 2005) result in the development of a permanent neurodegenerative disorder, known as "manganism" (Aschner et al., 2007; Hazell et al., 2006; Zheng et al., 2011). It is a motor syndrome resembling, but distinguishable from, idiopathic Parkinson's disease, and is characterized by the presence of Alzheimer type II astrocytosis, a distinctive feature of hepatic encephalopathy (Aschner et al., 2007; Hazell and Butterworth, 1999; Hazell et al., 2006; Layrargues et al., 1998; Normandin and Hazell, 2002; Orrenius et al., 2007).

Only a few reports have been published on the adverse effects of Mn toxicity on the liver, despite the fact that it is a major organ involved in regulating Mn homeostasis in the body. Acute Mn toxicity is unusual and is manifested primarily in the liver, presumably because of the primary role of this organ in removing Mn from the blood via biliary excretion (Crossgrove and Zheng,

Abbreviations: AChE, acetylcholinesterase; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BHT, butylated hydroxytoluene; CAT, catalase; CDNB, 1-chloro-2,4-dinitrobenzene; DTNB, 5,5-dithiobis-(2-nitrobenzoic acid); GSH, reduced glutathione; GST, glutathione *S*-transferase; LDH, lactate dehydrogenase; LPO, lipid peroxidation; Mn, manganese; NIH, National Institute of Health; ROS, reactive oxygen species; TBA, thiobarbituric acid; TBARS, thiobarbituric acid reactive substances.

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2004). Mn poisoning causes oxidative damage, changes in membrane fluidity, and histopathologic alterations in liver tissues (Huang et al., 2010, 2011); these changes reduce the rate of Mn excretion (Crossgrove and Zheng, 2004); this reduced excretion, in turn, results in an overflow of Mn from the liver to other organs, especially the brain (Huang et al., 1989). The cellular and molecular mechanisms underlying Mn-induced hepatotoxicity and/or neurotoxicity are believed to be numerous, but poorly understood (Dobson et al., 2004; Hazell and Butterworth, 1999; Milatovic et al., 2011). Mn is generally believed to exert cellular toxicity via a number of mechanisms, including the induction of free radical production; direct or indirect formation of reactive oxygen species (ROS) (Milatovic et al., 2007, 2011); changes in the functions of all neurotransmission pathways (Eriksson et al., 1992); and disruption of the homeostasis of Ca, Fe, and trace minerals (Chen et al., 2006; Zheng et al., 1999). An imbalance between ROS generation and antioxidant defense mechanisms results in oxidative stress (Chen et al., 2006), which can initiate apoptosis and/or necrosis in several tissues (Orrenius et al., 2007). Current approaches to minimize the severity of Mn toxicity include enhancement of its sequestration and elimination by using different treatment methods. Considering the relationship of Mn exposure with oxidative stress and elemental homeostasis, we can speculate that the administration of antioxidants and natural biomolecules may be protective in Mn toxicity. To our knowledge, an alternative approach for the management of Mn toxicity is yet to be discovered. This study on the effect of lycopene against Mn-induced neurological and hepatic impairment is the first step in this direction.

Lycopene, a bioactive compound normally found in red foods such as tomato, pink guava, watermelon, and papaya, has gained attention from nutritionists for its health benefits. It is a lipophilic carotenoid with 40 carbon atoms and is a highly unsaturated, straight, open-chain hydrocarbon containing 11 conjugated and 2 non-conjugated carbon–carbon (C=C) double bonds (Shi and Le Maguer, 2000). This extensive set of conjugated double bonds makes lycopene a potentially powerful antioxidant (Arab and Steck, 2000; Shi and Le Maguer, 2000), a characteristic believed to be responsible for its biogenic effects. The antioxidant activity of lycopene is highlighted by its singlet oxygen-quenching property and its ability to trap peroxyl radicals, contributing to the defense against lipid peroxidation (Kelkel et al., 2011; Kuhad et al., 2008; Shi and Le Maguer, 2000). From the several carotenoids that have been studied, lycopene has the strongest singlet oxygen-quenching ability, followed by α-carotene, β-carotene, and lutein (Di Mascio et al., 1989; Miller et al., 1996). This superior singlet-quenching ability of lycopene (relative to that of other carotenoids) is 2 times higher than that of β-carotene and 10 times higher than that of α-tocopherol (Agarwal and Rao, 2000). Lycopene has recently been reported to be associated with the decreased incidence of many important diseases including; cancer, atherosclerosis, age-related macular degeneration, and multiple sclerosis, probably via prevention of lipid peroxidation (Mortensen et al., 2001). As a natural antioxidant, lycopene has potent neuroprotective, antiproliferative, and anticancer activities (Hsiao et al., 2004; Kelkel et al., 2011). In addition, it has been reported to prevent liver fibrosis by inhibiting the activity of hepatic stellate cells (Kitade et al., 2002). However, to our knowledge, the protective effects of this carotenoid against Mn-induced neurohepatic toxicity have not yet been studied. Therefore, this study aimed to (1) characterize the neurohepatic changes induced by subacute exposure to Mn, i.e., (a) the levels of oxidative stress markers, trace minerals, and serum biochemical parameters and (b) the nature of histopathologic alterations; and (2) evaluate the possible protective effect of lycopene against Mn toxicity in rats.

2. Materials and methods

2.1. Chemicals, solvents and reagents

Mn chloride ($MnCl_2{\cdot}4H_2O$) was obtained from Fisher Scientific (Pittsburgh, PA); $MnCl_2{\cdot}4H_2O$ solution was freshly prepared by dissolving $MnCl_2$ in normal saline at a concentration of 6 mg Mn/ml saline. Atomic spectrophotometry standard solutions for Mn (as Mn nitrate in 3% nitric acid), iron (Fe in 3% nitric acid), and copper (Cu in 3% nitric acid) were purchased from Ricca Chemical Company (Fenton Mo). Reduced glutathione (GSH), thiobarbituric acid (TBA), and butylated hydroxytoluene (BHT) were purchased from Fluka (Buchs, Switzerland). Grade lycopene, nitric acid, 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB, Ellman's reagent), and 1-chloro-2,4-dinitrobenzene (CDNB) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All the other reagents were of analytical, high-performance liquid chromatography (HPLC), or the best available pharmaceutical grade.

2.2. Animals and experimental design

Thirty healthy adult male Sprague-Dawley rats, weighing 220 ± 7 g and aged 7–8 weeks, were purchased from the Medical Research Institute, Alexandria University, Egypt. All animals were housed in gang cages maintained in a room with controlled environment conditions and a 12-h light–dark cycle. The animal experiments were carried out in accordance with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals, and the study protocol was approved by the local authorities. All efforts were made to minimize the number of animals used and their suffering. After 2 weeks of acclimatization, all animals were randomly divided into 3 experimental groups of 10 rats each. Rats in the control or vehicles-received group were orally received corn oil for 20 days, then intraperitonealy (i.p.) injected with normal saline for 4 successive weeks; those in the Mn-treated group were orally received corn oil for 20 days, then i.p. injected with freshly prepared Mn solution at a dose of 6 mg Mn/kg bwt/day for 4 weeks; and those in the lycopene + Mn-treated group received a dose of 10 mg lycopene/kg bwt/day, orally dissolved in corn oil for 20 days before Mn treatment (dose same as that in the Mn-treated group) until the end of the experiment. The dose of lycopene used in the present study is based on reported doses which were found to be neuroprotective (Kumar et al., 2009; Sandhir et al., 2010).

Twenty-four hours after the last injection of saline or Mn, the rats were anesthetized with ketamine/xylazine (7.5:10 mg/kg, 1 mg/kg i.p.). Then, blood samples were collected from the inner canthus of the eye by heparinized capillary tube into 2 ml clean test tube. Following standing in room temperature for at least 30 min, the blood was centrifuged at $3400 \times g$ for 10 min at that point the serum was separated, transferred to eppendorf tubes, and stored at −20 °C prior to measure the concentrations of trace minerals, and determine other biochemical parameters. Immediately after the collection of blood samples, the animals were euthanized, and their livers and brains were quickly excised, rinsed in ice-cold saline and used immediately or stored frozen at −80 °C until analysis.

2.3. Tissue and serum trace minerals

200 mg tissues were weighed in a 10 ml microwave digestion vessels. An aliquot of 2 ml nitric acid was added into vessels. The tightly capped vessels were placed in hot air oven and digested with 15 min stepwise rise to 200 °C which was maintained for 10 min. After cooling to room temperature, the resulting solution was mixed with another 2 ml of distilled water. The solution was then transferred to 10 ml metal-free polypylene conical tube and