Food and Chemical Toxicology 55 (2013) 456–462

Contents lists available at SciVerse ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Mercuric chloride-induced testicular toxicity in rats and the protective role of sodium selenite and vitamin E

Suna Kalender [a,*], Fatma Gokce Uzun [b], Filiz Demir [b], Meltem Uzunhisarcıklı [c], Ayse Aslanturk [c]

[a] Gazi University, Gazi Education Faculty, Department of Science Education, Ankara, Turkey
[b] Gazi University, Faculty of Science, Department of Biology, Ankara, Turkey
[c] Gazi University, Vocational High School of Health Services, Ankara, Turkey

ARTICLE INFO

Article history:
Received 31 October 2012
Accepted 17 January 2013
Available online 29 January 2013

Keywords:
Mercuric chloride
Sodium selenite
Vitamin E
Histopathology
Oxidative stress
Testicular toxicity

ABSTRACT

Mercury has been recognized as an environmental pollutant that adversely affects male reproductive systems of animals. This study examined the effects of mercuric chloride on the antioxidant system and histopathological changes and also evaluated the ameliorating effects of sodium selenite and/or vitamin E in the rat testis tissues. Sexually mature male Wistar rats (weighing 300–320 g and each group six animals) were given mercuric chloride (1 mg/kg bw) and/or sodium selenite (0.25 mg/kg bw) + vitamin E (100 mg/kg) daily via gavage for 4 weeks. In the present study, mercuric chloride exposure resulted in an increase in the TBARS level and a decrease in the SOD, CAT, GPx activities, with respect to the control. Further, light microscopic investigation revealed that mercury exposure induced histopathological alterations in the testis tissues. Supplementation of sodium selenite and/or vitamin E to mercury-induced groups declined lipid peroxidation, increased SOD, CAT, GPx activities. While some histopathological changes were detected in mercuric chloride treated group, milder histopathological changes were observed in animal co-treated with sodium selenite and/or vitamin E supplementation to mercuric chloride-treated rats. As a result, mercuric chloride induced testicular toxicity is reduced by sodium selenite and/or vitamin E, but not ameliorate completely.

1. Introduction

Heavy metals have become one of toxic substances found in our environment (Orisakwe et al., 2001). Many of these metals are known to be extremely toxic (Orisakwe et al., 2001; Sharma et al., 2007a). In all heavy metals, mercury is one of the most widespread environmental pollutant, and it also used in industrial, pharmacological, agriculture and other fields (Mahboob et al., 2001; Sharma et al., 2007b).

Mercury, both inorganic mercury (mercuric chloride) and methyl mercury (MeHg) may cause accidental and occupational exposures and serious damage in various organs in human and experimental animals (Bando et al., 2005; Rao and Chhunchha, 2010). Inorganic mercury, well-established toxicant to human health, is found in the environment like water, food and air (Atkinson et al., 2001; Sharma et al., 2007a). Mercuric chloride is one of the most toxic forms of mercury because it easily forms organomercury complexes with proteins (Boujbiha et al., 2009). It is well known that hepatotoxic (Perottoni et al., 2004), neurotoxic (Franco et al., 2007), nephrotoxic (Sharma et al., 2007a), hematotoxic (Durak et al., 2010), genotoxic (Rozgaj et al., 2005) effects of inorganic mercury. In addition, it is reported that mercuric chloride has been adverse affect on reproductive system in experimental animals (Rao and Gangadharan, 2008; Boujbiha et al., 2011).

Heavy metal toxicity has been related with generation of reactive oxygen species (ROS). Increase of ROS usually leads to oxidative stress, which results cellular damage in different organs and tissues (Méndez-Armenta et al., 2011). Many studies show that mercuric chloride causes oxidative stress, characterized by exposure to excessive reactive oxygen species (ROS) (Su et al., 2008; Boujbiha et al., 2009; Rao and Chhunchha, 2010). The body has developed many defense mechanisms against oxidative damage. These defense mechanisms are enzymatic and non-enzymatic antioxidant systems (Eraslan et al., 2007). Antioxidant enzymes play an important role in mercury toxicity (Mahboob et al., 2001). Several reports have suggested that mercuric chloride enhance the production of ROS, and it alters the antioxidant enzyme activities in different tissues of rats (Bando et al., 2005; Boujbiha et al., 2009; Rao and Chhunchha, 2010).

Selenium (Se) is an essential element and well-establish antioxidant for all tissues of animals and human. In addition, it has various forms such as selenite, selenate, and it is important several

* Corresponding author. Address: Gazi University, Faculty of Gazi Education, Department of Science Education, 06500 Teknikokullar, Ankara, Turkey. Tel.: +90 312 202 82 27; fax: +90 312 222 84 83.
E-mail address: suna@gazi.edu.tr (S. Kalender).

0278-6915/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fct.2013.01.024

biochemical and physiological process (Su et al., 2008; Jihen et al., 2009; Sakr et al., 2011). Sodium selenite, a common dietary form of selenium, is recognized as essential in animal and human nutrition (Shilo et al., 2003). It is reported that sodium selenite has anticarcinogenic, antimutagenic properties (Geyikoglu and Turkez, 2005). Many studies have evaluated the protective effects of sodium selenite on mercury toxicity (Perottoni et al., 2004). Moreover, sodium selenite is commonly used as direct supplement for the treatment of selenium deficiency (Sakr et al., 2011). Recent evidences also show that selenium ameliorates oxidative damage by heavy metals (Su et al., 2008).

Vitamin E (alfa tocopherol), one of the most important vitamin for the body, is the major lipid soluble and well known non-enzymatic antioxidant (Al-Attar, 2011a; Patra et al., 2001; Uzun et al., 2009). Vitamin E inhibits peroxidation of membrane lipids by scavenging lipid peroxyl radicals (El-Demerdash et al., 2004; Uzunhisarcikli and Kalender, 2011), and it also inhibits oxidative damage in several tissues by heavy metals in experimental animals (Rao and Sharma, 2001; El-Demerdash et al., 2004; Acharya et al., 2008). It is reported that vitamin E protects the organism from toxic agent and free radical damage (Uzun et al., 2009; Kalender et al., 2010; Al-Attar, 2011b). It has been suggested that both Se and vitamin E has a protective role in peroxide damage to the sperm cell (Marin-Guzman et al., 2000). Therefore, it is indicated that both Se and vitamin E acts synergistically, and they also should be administered jointly (Kołodziej and Jacyno, 2005).

Male germ cells are more susceptible to oxidative stress than somatic cells, because of higher polyunsaturated fatty acids in their membranes (Acharya et al., 2008). Thus, the present study determined the possible adverse effects of mercuric chloride on testicular parameters, and to assess whether these affects can be ameliorated by co-treatment with sodium selenite and vitamin E.

2. Materials and methods

2.1. Animals

Wistar albino male rats (weighing approximately 300–320 g) were obtained from the Gazi University Laboratory Animals Growing and Experimental Research Center was used. Animals were housed in plastic cages under a well-regulated light–dark (12 h:12 h) schedule at 22 + 3 and fed with standard laboratory diet and top water *ad libitum*. The animals were quarantined for 10 days before beginning the experiment. All rats were handles in accordance with the standards guide for the care and use of laboratory animals. The Gazi University Committee on the Ethics of Animal Experimentation approved all animal experiments.

2.2. Chemicals

Mercuric chloride (99% purity) and sodium selenite (99% purity) were supplied by Sigma Aldrich (Germany). Vitamin E was supplied by Merck (Germany).

2.3. Experimental protocols

Rats were divided eight groups, each consisting of six rats. Group 1 – control rats (treated with 1 mg/kg bw corn oil per day); Group 2 – sodium selenite treated rats (0.25 mg/kg bw per day in distilled water); Group 3 – vitamin E treated rats (100 mg/kg bw per day in corn oil); Group 4 – vitamin E plus sodium selenite treated rats (100 mg/kg bw + 0.25 mg/kg bw per day, respectively); Group 5 – mercuric chloride treated rats (1 mg/kg bw per day in distilled water); Group 6 – sodium selenite plus mercuric chloride (0.25 mg/kg bw + 1 mg/kg bw per day, respectively); Group 7 – vitamin E plus mercuric chloride (100 mg/kg bw + 1 mg/kg bw per day, respectively); Group 8 – sodium selenite plus vitamin E plus mercuric chloride (0.25 mg/kg + 100 mg/kg bw + 1 mg/kg bw per day, respectively). The doses were chosen by the basis of previous studies (Ramalingam and Vimaladevi, 2002; El-Demerdash et al., 2004; Koyuturk et al., 2007). The substances were administrated in the morning (between 09:00 and 10:00 h) to non-fasted rats. The first day the animals were treated was considered experimental Day 0. At the end of the 4th week (28 days) of treatment, all animals were sacrificed and dissected. The testis tissues were quickly excised to light microscope investigations and biochemical examinations.

2.4. Biochemical estimation

The testis tissues were dissected and washed in sodium phosphate buffer (pH 7.2). After washing, samples were taken and store at −80 °C until analysis. The tissues were homogenized using a Teflon homogenizer (Heidolph Silent Crusher M), and then the homogenates were centrifuged. Thiobarbituric-acid-reactive species (TBARS) content and antioxidant enzyme activities were determined by measuring the absorbance of the samples in a spectrophotometer (Shimadzu UV 1700, Kyoto, Japan). Protein content of supernatant was determined by the method of Lowry et al., (1951) using bovine serum albumin as standard.

2.4.1. Lipid peroxidation assay

TBARS content was evaluated using the thiobarbituric acid (TBA) test as described by Ohkawa et al. (1979). After incubation of testis homogenate with TBA at 95 °C, TBARS reacts to form a colored complex. Absorbance was measured spectrophotometrically at 532 nm to determine the TBARS content. The specific activity is expressed as nmol/mg protein protein.

2.4.2. Measurement of superoxide dismutase (SOD)

SOD activity was measured according to the method described by Marklund and Marklund (1974) by assaying the autooxidation and illumination of pyrogallol at 440 nm for 3 min. One unit of SOD activity was calculated as the amount of protein that caused 50% pyrogallol autooxidation inhibition. A blank without homogenate was used as a control for non-enzymatic oxidation of pyrogallol in Tris–EDTA buffer (50 Mm Tris, 10 mM EDTA, pH 8.2). The SOD activity is expressed as U/mg protein.

2.4.3. Measurement of catalase (CAT)

CAT activity was measured determined according to the method described by Aebi (1984) by assaying the hydrolysis of H_2O_2 and the resulting decrease in absorbance at 240 nm over a 3 min period at 25 °C. Before determination of the CAT activity, samples were diluted 1:9 with 1% (v/v) Triton X-100. CAT activity is expressed as mmol/mg protein.

2.4.4. Measurement of glutathione peroxidase (GPx)

GPx activity was measured using H_2O_2 as substrate according to the method described by Paglia and Valentine (1967). The reaction was monitored indirectly as the oxidation rate of NADPH at 240 nm for 3 min. A blank without homogenate was used as a control for non-enzymatic oxidation of NADPH upon addition of hydrogen peroxide in 0.1 M Tris buffer, pH 8.0. Enzyme activity was expressed as nmol/mg protein.

2.5. Histopathology

For histopathological examination, testis tissues were dissected and fixed in Bouin solution. Then samples were processed using a graded ethanol series, and embedded in paraffin. The paraffin sections were cut into 6–7 μm-thick slices and stained with hematoxylin and eosin for histological examination. The sections were viewed and photographed using an Olympus light microscope (Olympus BX51, Tokyo, Japan) with an attached camera (Olympus E-330, Olympus Optical Co. Ltd., Japan).

2.6. Statistical analysis

The data were analyzed by using SPSS 11.0 for Windows. Statistical significance of difference was evaluated by using one-way analysis of variance (ANOVA) followed by Tukey's procedure for multiple comparisons. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Evaluation of biochemical parameters

At the end of the experiment, there were no statistically significant differences in TBARS levels or SOD, CAT, GPx activities between the Group 2, Group 3 and Group 4 compared to Group 1 ($P < 0.05$, Figs. 1–4).

3.1.1. Thiobarbituric-acid-reactive species (TBARS) levels

TBARS level was significant increase in Group 5, Group 6, Group 7 and Group 8 with respect to Group 1, Group 2, Group 3 and Group 4 rats. Whereas, a significant decline in TBARS level was noticed in combined treatment of Group 6, Group7 and Group 8 compared to Group 5 ($P < 0.05$, Fig. 1).