Food and Chemical Toxicology 70 (2014) 185–190

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

Sodium selenite and vitamin E in preventing mercuric chloride induced renal toxicity in rats

Ayse Aslanturk [a,*], Meltem Uzunhisarcikli [a], Suna Kalender [b], Filiz Demir [c]

[a] Gazi University, Vocational High School of Health Services, Ankara, Turkey
[b] Gazi University, Gazi Education Faculty, Department of Science Education, Ankara, Turkey
[c] Gaziosmanpaşa University, Graduate School of Natural and Applied Sciences, Tokat, Turkey

ARTICLE INFO

Article history:
Received 14 January 2014
Accepted 15 May 2014
Available online 22 May 2014

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

ABSTRACT

This study aims to investigate improving effects of sodium selenite and/or vitamin E on mercuric chloride-induced kidney impairments in rats. Wistar male rats were exposed either to sodium selenite (0.25 mg/kg day), vitamin E (100 mg/kg day), sodium selenite + vitamin E, mercuric chloride (1 mg/kg day), sodium selenite + mercuric chloride, vitamin E + mercuric chloride and sodium selenite + vitamin E + mercuric chloride for 4 weeks. Mercuric chloride exposure resulted in an increase in the uric acid, creatinine, blood urea nitrogen and malondialdehyde (MDA) levels and a decrease in the superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities. Histopathological changes were detected in kidney tissues in mercuric chloride-treated groups. A significant decrease in the uric acid, creatinine, blood urea nitrogen and MDA levels and a significant increase in the SOD, CAT and GPx activities were observed in the supplementation of sodium selenite and/or vitamin E to mercuric chloride-treated groups.

Conclusively, sodium selenite, vitamin E and vitamin E + sodium selenite significantly reduce mercuric chloride induced nephrotoxicity in rats, but not protect completely.

1. Introduction

Industrial pollution of the environment with metal compounds is becoming an important problem (El-Shenawy and Hassan, 2008). Mercury is a widespread environmental and industrial pollutant. It is known that mercury may cause accidental and occupational exposures and consequential damage in various organs in human and animals (Agarwal et al., 2010; Rao and Chhunchha, 2010). People can be exposed to mercury through contaminated water and food (Magos and Clarkson, 2006). Poisoning can result from inhalation, ingestion and absorption through the skin (El-Shenawy and Hassan, 2008). The kidney, liver, gastrointestinal system and central nervous system are the main target sites of mercury toxicity (Agarwal and Behari, 2007). Mercuric chloride ($HgCl_2$) is a potent nephrotoxic agent that has been widely used in animal models for studying acute renal failure because it causes oxidative stress and renal damage (Haibo et al., 2011).

The toxic effects of divalent mercury can be prevented by some extent antioxidant defence mechanisms (Pillai and Gupta, 2005). Selenium is considered as an essential trace mineral for living organisms, because it is a structural component of several enzymes like glutathione peroxidases and thioredoxin reductase (Su et al., 2008; Agarwal and Behari, 2007). Selenium has been found to have detoxification effects on various heavy metals (Diplock et al., 1986). Vitamin E is a lipid–soluble vitamin, which plays an important role in neutralizing the toxic effects of reactive oxygen species and alpha-tocopherol is the most reactive form of vitamin E (Stocker et al., 1991; Navarro et al., 1999; Warren et al., 2000; Uzunhisarcikli and Kalender, 2011). Vitamin E protects the organism from free radical damage (Kalender et al., 2010; Al-Attar, 2011). The above studies indicate that vitamin E and selenium are hopeful due to their antiradical activities and they could provide a significant dietary source of antioxidants (Amara et al., 2011).

The present study has been designed to evaluate the protective effect of sodium selenite and/or vitamin E treatment on kidney tissue of mercuric chloride intoxicated rats. Blood urea nitrogen, uric acid and creatinine levels were studied to assess the kidney functions. Lipid peroxidation and antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase activities

* Corresponding author. Address: Gazi University, Vocational High School of Health Services, Gölbaşı, Ankara, Turkey. Tel.: +90 312 484 11 25; fax: +90 312 484 36 49.
E-mail addresses: aogutcu@gazi.edu.tr, ayseogutcu@gmail.com (A. Aslanturk).

http://dx.doi.org/10.1016/j.fct.2014.05.010
0278-6915/

were determined to evaluate oxidative stress and also, histopathological changes were examined in rat kidney tissue as indicators of mercuric chloride exposure.

2. Materials and methods

2.1. Chemicals

Mercuric chloride (99% purity) and sodium selenite (99% purity) were obtained from Sigma Aldrich (Germany). Vitamin E (DL-α-tocopherol acetate; 500 mg DL-α-tocopherol acetate per ml) was supplied by Merck (Germany).

2.2. Animals, experimental design and collection of tissues

Forty eight Wistar albino male rats (weighing 300–320 g) from the Gazi University Laboratory Animals Growing and Experimental Research Center were maintained at the controlled temperature (22 ± 3) and 12 h alternate light and dark conditions and fed with standard laboratory diet and top water *ad libitum*. All animal experiments were approved by the Gazi University Committee on the Ethics of Animal Experimentation (G.U. ET – 10.026).

Ten days after acclimatization to laboratory conditions, rats were randomly divide into eight groups, each consisting of six rats. Group 1 served as control (treated with 1 mg/kg bw corn oil per day); Group 2 received sodium selenite (0.25 mg/kg bw per day in distilled water); Group 3 was treated vitamin E (100 mg/kg bw per day in corn oil); Group 4 received vitamin E + sodium selenite (100 mg/kg bw + 0.25 mg/kg bw per day, respectively); Group 5 received mercuric chloride (1 mg/kg bw per day in distilled water); Group 6 was treated with sodium selenite + mercuric chloride (0.25 mg/kg bw + 1 mg/kg bw per day, respectively); Group 7 received both vitamin E and mercuric chloride (100 mg/kg bw + 1 mg/kg bw per day, respectively); Group 8 was treated sodium selenite + vitamin E + mercuric chloride (0.25 mg/kg + 100 mg/kg bw + 1 mg/kg bw per day, respectively). Rats were treated orally with these compounds for 4 weeks.

Rats were sacrificed under anesthesia at the end of the treatment period. Blood was drawn from the heart in non-heparinized (to measure blood urea nitrogen, uric acid, creatinine). The kidney tissues were removed immediately for light microscope investigations and biochemical assays and weighed by employing automatic balance (AND GX-600, Japan). The kidney tissues were dissected and washed in sodium phosphate buffer (pH 7.2). Kidney tissues were homogenized using a Teflon homogenizer (Heidolph Silent Crusher M). Homogenates were centrifuged. The resulting supernatants were used for estimation lipid peroxidation and antioxidant enzyme activities. Moreover, kidney tissues also after were fixed in Bouin solution for histopathological examination.

2.3. Biochemical assays

2.3.1. Assessment of oxidative stress

Malondialdehyde (MDA) content was determined using the thiobarbituric acid (TBA) test as described by Ohkawa et al., 1979. Absorbance was measured at 532 nm to evaluate the MDA content. The specific activity was expressed as nmol/mg protein.

Superoxide dismutase (SOD) activity was estimated according to 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. The SOD activity was expressed as U/mg protein.

Catalase (CAT) activity was assayed by the method of 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. CAT activity was expressed as mmol/mg protein.

Glutathione peroxidase (GPx) activity was performed using H_2O_2 as substrate according to Paglia and Valentine (1967). The reaction was monitored indirectly as the oxidation rate of NADPH at 240 nm for 3 min. The enzyme activity was expressed as nmol/mg protein.

Protein was assayed according to the method of Lowry et al. (1951) using bovine serum albumin (BSA) as standard.

2.3.2. Assessment of renal functions

Blood urea nitrogen, uric acid, creatinine were assessed in serum using a commercially available kit (Roche Diagnostics GmbH, D-68298, Mannheim) and analyzed by autoanalyzer (Roche Diagnostics Cobas Integra 800, Mannheim).

2.4. Histopathology

The kidney tissues were dissected and fixed in Bouin solution and then processed using a graded ethanol series and embedded in paraffin for histopathological studies. Sections of 6–7 μm thickness were cut and stained with hematoxylin and eosin. 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).

2.5. Statistical analysis

Data of the present study were analyzed using SPSS 15.0 for Windows. Statistical significance of difference in values control and treated animals was calculated using one-way analysis of variance (ANOVA) followed by Tukey's procedure for multiple comparisons. All values were expressed as means ± S.D. Differences were considered significant if $p < 0.05$.

3. Results

3.1. Evaluation of absolute and relative kidney weights

At the end of the 4th week, no statistically significant changes were observed between the control group, sodium selenite-treated group, vitamin E-treated group, and vitamin E plus sodium selenite-treated group rats in terms of absolute and relative kidney weights (Table 1).

Absolute kidney weight significantly decreased in the mercuric chloride-treated group, sodium selenite plus mercuric chloride-treated group, vitamin E plus mercuric chloride-treated group, sodium selenite plus vitamin E plus mercuric chloride-treated group rats compared to control group, sodium selenite-treated group, vitamin E-treated group, and vitamin E plus sodium selenite-treated group rats. There was no change in relative kidney weight ($p < 0.05$) (Table 1). No statistically significant changes were observed between mercuric chloride-treated group, sodium selenite plus mercuric chloride-treated group, vitamin E plus mercuric chloride-treated group, sodium selenite plus vitamin E plus mercuric chloride-treated group rats in terms of absolute and relative kidney weights ($p < 0.05$), (Table 1).

3.2. Evaluation of oxidative stress

No significant alterations in MDA level and SOD, CAT, GPx activities were observed in the sodium selenite-treated group, vitamin E-treated group, and vitamin E plus sodium selenite-treated group rats compared to the control group rats ($p < 0.05$, Table 2).

MDA level significantly increased while SOD, CAT, GPx activities significantly declined in all mercuric chloride intoxicated groups. There was statistically significant reduction in MDA level and an increase in the SOD, CAT, GPx activities in the sodium selenite plus mercuric chloride-treated group, vitamin E plus mercuric chloride treated group, sodium selenite plus vitamin E plus mercuric chloride-treated group rats compared to the mercuric chloride-treated group rats ($p < 0.05$, Table 2).

3.3. Evaluation of renal functions

Blood urea nitrogen, uric acid and creatinine levels were studied to assess the renal functions. Results showed no significant differences between the sodium selenite-treated group, vitamin E-treated group, vitamin E plus sodium selenite-treated group rats and the control group rat with regard to blood urea nitrogen, uric acid and creatinine levels ($p < 0.05$, Table 2).

At the end of the 4th week when the mercuric chloride-treated group, sodium selenite plus mercuric chloride-treated group, vitamin E plus mercuric chloride-treated group, sodium selenite plus vitamin E plus mercuric chloride-treated group rats were compared with the control group rats, there was a significant increase in the blood urea nitrogen, uric acid and creatinine levels. The blood urea nitrogen, uric acid and creatinine levels significantly decreased at the end of the 4th week in the sodium selenite plus mercuric chloride-treated group, vitamin E plus mercuric chloride-treated group, sodium selenite plus vitamin E plus mercuric chloride-treated group rats compared to the mercuric chloride-treated group rats ($p < 0.05$, Table 2).