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Toxicology

Protective effect of omega-3 fatty acid against mercury chloride intoxication in mice

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ABSTRACT

The aim of this study was to investigate the protective effect of omega-3 fatty acid in $HgCl_2$ toxicity in mice. Levels of malondialdehyde (MDA), reduced glutathione (GSH), nitric oxide (NO) and total sialic acid (TSA), and histopathological changes in selected organs were evaluated. Twenty-eight mice were equally divided into 4 groups, namely Groups I–IV. Group I animals received intraperitoneal (ip) injection of physiological saline solution; Group II animals received ip injection of 0.4 mg/kg/day $HgCl_2$; Group III animals received ip injection of 0.4 mg/kg/day $HgCl_2$ in addition to subcutaneous (sc) injection of 0.5 g/kg/day omega-3 fatty acid; and Group IV animals received sc injection of 0.5 g/kg/day omega-3 fatty acid. All treatments lasted 7 days. The levels of MDA, NO and TSA were significantly higher in Group II and lower in Groups III and IV as compared to the Group I. GSH level was the highest in Group IV. In histopathology, severe degeneration in liver and kidney was observed in Group II animals. These degrading changes were seen to be reduced greatly in Group III animals. The results suggested that omega-3 fatty acid might attenuate $HgCl_2$-induced toxicity by improving antioxidant status and acute phase response in mice.

Introduction

Mercury is a well-known toxic heavy metal to animals as well as humans. It is widespread and distributed throughout the environment by countless processes such as volcanic activities, water movements and various biological activities. Industrial processes such as mining activities, combustion of fossil fuels, pesticides and medical wastes have also become significant contributors to the environmental distribution of mercury and its compounds [1,2].

Mercury causes various metabolic changes as a result of its toxic effects especially in central nervous system, kidney and liver tissues of animals. It manifests its toxicity by interacting with reactive oxygen species (ROS) and binding to thiol groups in several proteins such as glutathione (GSH) and many of the antioxidant enzymes [3]. High levels of ROS, which indicates the oxidative stress, are known to induce lipid peroxidation that can be further shown by increased malondialdehyde (MDA) levels [4,5]. Nitric oxide (NO) is involved in various biological processes such as vasodilatation, neurotransmission and leukocyte mediated killing of pathogens. Moreover, it is involved in the process of peroxynitrite anion ($ONOO^-$) production reacting with peroxides, which further yields lipid peroxidation [6]. Total sialic acid (TSA) can be used as an indicator of tissue damage. Sialic acids have many important physiological and pathological functions such as cellular transmission, embryogenesis, organ development, immune system regulations, leucodiapedesis, metastasis of neoplastic cells, and carrying out membrane receptor functions [7–10].

The omega-3 fatty acids are polyunsaturated fatty acids and commonly found in marine oils. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) compose of the major parts of omega-3 fatty acids. In recent years, it has been reported that consumption of long-chain omega-3 fatty acids may have beneficial effects in many health problems such as cardiovascular, neuronal and gastrointestinal diseases related particularly to electrical signal modulation [11,12]. In the present study, potential protective effect of omega-3 fatty acids in $HgCl_2$ toxicity in mice was investigated by biochemical and histopathological means.

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Materials and methods

Experimental design

The ethical approval of the study was confirmed by İnönü University Animal Care and Use Committee (Registration Number: 2013/A-41). All procedures were conducted in accordance with the 'Guide for Care and Use of Laboratory Animals' published by the National Institutes of Health and the ethical guidelines of the International Association for the Study of Pain.

Experiments were carried out on 28 male Swiss albino mice each weighing 29–34 g at 14–16 weeks of age. Animals were divided into 4 equal groups namely Group I through Group IV and housed in a room maintained at 18 ± 1 °C with an alternating 12 h light-dark cycle. Food and water were provided *ad libitum*. All experimental injections were carried out for 7 days and at the same hours of the light cycles of the study. Treatments were performed as follow; Group I: ip injection of physiological saline solution, Group II: ip injection of 0.4 mg/kg/day $HgCl_2$. Group III: ip injection of 0.4 mg/kg/day $HgCl_2$ plus sc injection of 0.5 g/kg/day omega-3 fatty acid, and Group IV: sc injection of 0.5 g/kg/day omega-3 fatty acid.

Biochemical analysis

At the end of the 7th day, blood samples were collected into EDTA tubes from the hearts *via* cardiac puncture under ether anesthesia for GSH, MDA and NO measurements. The blood samples were centrifuged (1200 × *g*, 4 °C) for 10 min, and the plasma were obtained and then kept at −25 °C until the analyzes were carried out. At necropsy liver, kidney and brain tissues were collected to determine tissue GSH, MDA, NO and TSA concentrations. For this purpose, the tissues were rinsed with ice-cold 0.9% NaCl. Then, 1 g of tissue was homogenized in phosphate buffer (pH 7.4) in an ice bath and the homogenates were centrifuged (1200 × *g*, 4 °C) for 15 min.

TSA was measured calorimetrically using a spectrophotometer (UV-1201, Shimadzu, Japan) by the method of Sydow [13] in that all bound sialic acid were separated by acid per-chloride in plasma and tissue homogenates, and then the supernatants were boiled by Erlich reagent, and finally the product was read at 525 nm.

GSH concentration was assayed by the method of Beutler et al. [14] based on the spectrophotometric measurement of sulphydril (—SH) groups forming complexes with 5,5′-(2-dithiobis nitrobenzoic acid) which give rise to colored products which were read at 412 nm.

Measurement of MDA concentrations was carried out by the method of Yoshoiko et al. [15] based on the reaction between thiobarbituric acid and MDA produced as an end product of lipid peroxidation. The end products were read at 535 nm.

NO levels were determined according to the method described by Miranda et al. [16] in that nitrate is reduced to nitrite by $VaCl_3$, and then in acidic environment nitrite was reacted with sulphanilamide to produce colored diazonium compound, which was read at 540 nm.

Histopathological investigations

Tissue sections of liver, kidney and brain were sliced and fixed in 10% phosphate buffered formalin. After the specimens were dehydrated in serial ethanol and xylene, they were embedded in paraffin. Tissue sections were then cut and processed for hematoxylin and eosin (HE) staining. The sections were examined unbiased under a light microscope.

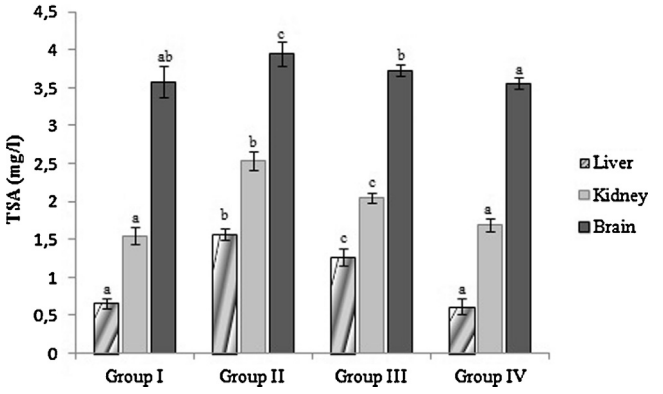

Fig. 1. Levels of the liver, kidney and brain TSA in $HgCl_2$ and omega-3 fatty acids-treated and without treated groups. Results with different superscripts within the same row are significantly different ($P < 0.001$).

Statistical analysis

SPSS software (Windows version 20.0) was used for statistical evaluation of data which were expressed as median (X) ± standard deviation (SD). Importance level of difference among the groups was determined by variance analysis (ANOVA) and Duncan multiple comparison test.

Results

TSA, MDA, GSH and NO levels

The results of TSA, MDA, GSH and NO levels in liver, kidney, and brain tissues were shown in Figs. 1–4 respectively. Blood MDA, GSH and NO levels were also shown in Fig. 5. The levels of MDA and NO in liver, kidney, brain and blood tissues were significantly higher in mice received $HgCl_2$ alone compared to that of Group I animals used as control. On the other hand, compared to the control group, the levels of MDA and NO were lower in mice received omega-3 fatty acid alone. Comparably, the levels of TSA in liver, kidney and brain tissues were significantly higher in Group II compared to Group I. Contrary to TSA, MDA and NO, GSH levels were significantly lower in liver, brain and blood, while no change was detected in kidney. In liver, omega-3 fatty acid injection in $HgCl_2$ received mice had significant effects by decreasing TSA, GSH and NO levels and increasing GSH level compared to that of mice received $HgCl_2$ alone. Similar results were also observed for brain and blood. In kidney, GSH levels were comparable among the groups, however TSA, MDA

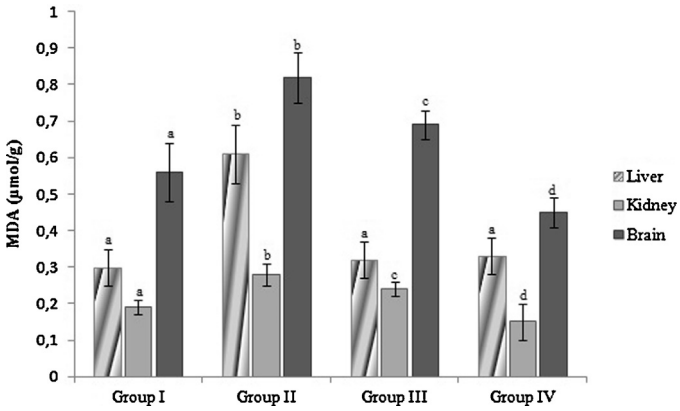

Fig. 2. Levels of the liver, kidney and brain MDA in $HgCl_2$ and omega-3 fatty acids-treated and without treated groups. Results with different superscripts within the same row are significantly different ($P < 0.001$).