Original Article

Effects of Vitamin C on Paraoxonase1 Arylesterase Activity in Rats Exposed to Arsenic

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ABSTRACT

Background: Arsenic as an environmental toxicant is able to induced oxidative stress. The present study aimed to evaluate of vitamin C supplementation on the paraoxonase1 (PON1) arylesterase activity (responsible for hydrolysing lipid-peroxides) in rat exposed to arsenic.

Methods: The study was conducted in the Faculty of Veterinary Medicine, Tabriz Islamic Azad University, Tabriz, Iran in 20015. Thirty-two adult male rats weighing 200-350 g were randomly divided into 4 groups: group I (Normal healthy rat), group II [treated with sodium arsenite (100 ppm in drinking water)], group III [treated with vitamin C supplementation (200 mg/kg via gavage)] group IV (treated with sodium arsenite and vitamin c 100 ppm and 200 mg/kg respectively).

Results: Vitamin C supplementation increased paraoxonase1 (PON1) arylesterase activity (P<0.05) but had no significant effect on serum levels of HDL, triglyceride and cholesterol**.**

Conclusion: PON1 arylesterase activity is independent of changes in HDL and lipid profile, and vitamin C with different mechanisms such as radical scavenging activity and reduction of oxidative stress leads to increased activity of paraoxonase.

Keywords: Arsenic, Paraoxonase 1, Rat, Vitamin C.

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INTRODUCTION

Arsenic is one of the most toxic metalloids, found in soil, water and air. Toxic effects of arsenic are attributed to the production of reactive oxygen species (ROS) and oxidative stress, resulting in the alteration of the antioxidant defense system, increased oxidative stress and cell death (1, 2). Oxidative stress has been implicated in various pathological conditions such as cardiovascular disease (3, 4). Paraoxonase (PON1) is an enzyme produced in the liver, associated with HDL (High-density lipoprotein) and has an antioxidant effect (5).

It hydrolyzeds organophosphates such as paraoxon and aromatic esters such as phenyl acetate and reduces the accumulation of peroxidation products (6). It has paraoxonase and arylesterase (ARE) activity. PON1 and ARE are both enzymes that have antioxidant and lipophylic properties. These two enzymes are important in reducing oxidative stress (7, 8). PON1 is scavenging of free radicals in the human and has protective effects against cardiovascular disease (6, 9). Studies have shown the significant relationship between arsenic exposure and PON1 activity in atherosclerotic patients (10). Vitamin C is a water-soluble vitamin with antioxidant activity that reduces the damage caused by free radicals (11, 12). A number of studies have addressed the beneficial effects of vitamin C on PON1 enzyme activity (13, 14).

The aim of this study was to investigate the effects of vitamin C supplementation on the arylesterase activity of PON1 and lipid profile in rats exposed to arsenic.

MATERIALS AND METHODS

Animal and Experimental Groups

The study was conducted in the Faculty of Veterinary Medicine, Tabriz Islamic Azad University, Tabriz, Iran in 20015. Thirty-two adult male rats weighing 200-350 g were randomly divided into 4 groups: group I (Normal healthy rat), group II [treated with sodium arsenite (100 ppm in drinking water)], group III [treated with vitamin C supplementation (200 mg/kg via gavage)] group IV (treated with sodium arsenite and vitamin c 100 ppm and 200 mg/kg respectively). The groups were treated every day for 30 days.

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The experimental protocol was approved by the Medical Ethics Committee, and all animals received humane care in compliance with the guidelines of Tabriz University of Medical Sciences.

Arylesterase Activity Assay

ARE activity was measured using phenylacetate as the substrate. The assay tube contained 750 µL of 0.1 mol/L Tris-HCl at pH 8.5, 1 mmol/L $CaCl_2$, 125 µL of 12 mmol/L phenylacetate, and 125 µL of serum (diluted 1:10 with water). The increase in absorbance was continuously recorded at 270 nm and 37 ºC. The units were expressed as millimoles of phenylacetate hydrolyzed per minute (8).

Lipid Profile Assay

Serum lipids (total cholesterol, triglycerides, and HDL) were determined by Abbott ALCYONTM300 Autoanalyzer, enzymatic methods using Kits (Pars Azmoon, Iran) according to the manufacture's instruction LDL levels (low density lipoprotein) were calculated by the formula of Friedewald: [LDL-cholesterol] = [total Cholesterol] - [HDL-cholesterol] - [Triglycerides]/5.

Malondialdehyde (MDA) Assay

Malondialdehyde (MDA) [marker of lipid peroxidation] levels were measured using the tiobarbituric acid (TAB) [15]. It was expressed as nmol/mg protein in tissue, nmol/ml in serum.

Statistical Analysis:

The mean value ±SD was determined for each variable in all groups. Data were fed into SPSS, 16.0 (Chicago, IL, USA). Mann Whitney U test was used to compare the groups at the significance level $P<0.05$.

RESULTS

The effects of vitamin C on PON1arylesterase activity, lipid profile and MDA (Malondialdehyde) are shown in Table 1.

Vitamin C supplementation increased PON1 arylesterase activity ($P<0.05$) but had no significant effect on serum levels of HDL-C, triglyceride and cholesterol.

Table 1. Effects of vitamin C on PON1 arylesterase activity, MDA and lipid profile in all groups.

parameters	Group I	Group II	Group III	Group IV
MAD (nmol/ml)	3/07±0.51	3.78±0.70*	2.38±0.46	2.76±0.41**
PON1arylesterase activity (U/L)	62.7±7.52	38.43±4.64*	71.40±7.91	54.56±5.43**
Cholesterol (mg/dl)	84.50±13.47	81.33±8.86	76.60±9.01	73.17±9.74
HDL (mg/dl)	38±2.19	36.67±4.13	41.4±6.98	40±5.79
LDL (mg/dl)	33.66±11.85	35.56±6.88*	26.56±12.37	24.96±7/01**
Triglyceride (mg/dl)	43.17±4.07	44.17±8.23	42.20±9.32	41±9.33

* $P<0.05$ compared to group I
** $P<0.05$ compared to group II

DISCUSSION

Serum levels of MDA and LDL-C significantly increased in the rats exposed to sodium arsenite ($P<0.05$). A significant decrease was seen after vitamin C treatment in serum MDA and LDL-C ($P<0.05$). Arsenic was a potent inducer of lipid peroxidation (1). These results are in agreement with the findings of several studies (12, 16). The increase of MDA could be related to the GSH (glutathione) depletion. GSH serves as an essential antioxidant molecule responsible for metabolism and detoxification of xenobiotics such as arsenic. Lipid peroxidation, one of the mechanisms of arsenic toxicity, causes a decrease in cellular GSH concentration, which is inversely correlated with lipid peroxidation (17-19).

Vitamin C is a water-soluble antioxidant, which acts as a free radical chain terminator. This vitamin protects the cells from reactive oxygen species (ROS) and inhibits the oxidation of lipids. Vitamin C can form a complex with heavy metals. Its use in rats exposed to arsenic may have a protective effect (20, 25). As indicated in Table 1, the lipid profile (cholesterol, triglyceride and HDL-C) did not show a significant change in response to vitamin C, which is consistent with previous studies (12, 25, 26). One possible reason for this finding is that levels of vitamin C are not sufficient to have positive effects on HDL-C. In addition, some of studies have shown that arsenic in drinking water can cause different of dyslipidemia (16).

Increases of plasma concentrations of free fatty acids inhibit the enzyme lipoprotein lipase (LPL) so that it can cause lead to hypertriglyceridemia and hypercholesterolemia and decrease of HDL-C (16).

A significant reduction (P<0.05) in LDL-C was seen after using vitamin C in rats exposed to arsenic. This may be due to activation of 7-α-hydroxylase enzyme by vitamin C, which enhances the conversion of cholesterol to bile acids and thus reduce the serum levels of cholesterol. Long-term use of vitamin C significantly reduce total cholesterol and LDL-Cand no significant effect on the reduction of VLDL and triglycerides (12, 24). The results of the present study show that arsenic reduced arylesterase activity of PON1. Vitamin C supplementation increased arylesterase activity of this enzyme in rats exposed to arsenic, which is consistent with earlier studies (13, 14, 27). PON1 is an HDL- associated antioxidant in human plasma and easily inactivated by the endogenous and exogenous antioxidants such as arsenic and OX-LDL is disabled. Vitamin C may increase PON1 activity, leading to a reduction in the OX-LDL (10, 28, 29). Thus, the assessment of arylesterase activity of this enzyme may be useful in diseases related to low activity of that enzyme such as cardiovascular disease (10, 28).

CONCLUSION

This study addressed the effect of vitamin C on PON1 arylesterase activity. It can be replicated by considering other conditions like the use of other oxidants and heavy metals, different doses and different forms of arsenic, rout of arsenic and vitamin C. Vitamin C supplementation resulted in an increase in PON1arylesterase activity as well as in decrease in serum LDL-C.

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