IJPT
1735-2657/10/92-69-71
IRANIAN JOURNAL OF PHARMACOLOGY & THERAPEUTICS

IJPT 9: 69-71, 2010

RIDR

RESEARCH ARTICLE

Changes in Biochemical Parameters Related to Lipid Metabolism Following Titanium Treatment in Rat

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Received December 6, 2009; Accepted July 4, 2010

This paper is available online at *http://ijpt.iums.ac.ir*

ABSTRACT

Titanium salts are widely used in pharmacy and industry as intensely white permanent pigment with good covering power in paints, paper, toothpaste, plastics and food dyes. This ion is a redox-active transition metal and may cause oxidative damages to lipids, proteins and DNA molecules. In spite of abundant literature, questions about the side effects of titanium ions still remain to be answered. In this study, the effects of titanium on biochemical parameters related to lipid metabolism were investigated. Male Wistar rats were treated with different doses of titanium for a period of up to 60 days. Blood samples were then collected for analysis. Lipid-related parameters in plasma were measured by standard methods. It was shown that titanium reduced plasma VLDL and triglycerides concentrations but increase in LDL-C and cholesterol levels were seen in all experimental groups. Titanium also showed to inhibit lipoprotein lipase activity. The finding that titanium increases LDL and cholesterol concentrations should be considered seriously, especially in people exposing to titanium compounds for a long period.

Keywords: *Titanium, Lipid parameters, Rat*

Hypercholesterolemia is a risk factor for the development of many diseases including atherosclerosis, myocardial infarction, heart attacks, and cerebral paralysis [1,2]. Thus, the normalization of serum levels of total cholesterol or low-density lipoprotein cholesterol (LDL-C) through diet therapy or drug administration has been shown to decrease the incidence of coronary heart disease [3-5]. Agents that interfere with lipid metabolism may increase the susceptibility to atherosclerosis and are the subjects of widespread investigations.

Titanium is widely used in industry as a white permanent pigment with good covering power in paints, paper, toothpaste, plastics and food dyes[6-8]. It is also used as components of large systems, such as cement, dielectric mirrors, cosmetic and skin care products, surgical instruments, dental implants, pace-makers, bone-plates and screws and many other alloy products. Therefore, studies on the effects of titanium intake on body metabolism are of especial interest [9-13]. In vitro Studies have demonstrated that similar to iron and copper, titanium acts as a catalyst in the formation of reactive oxygen species (ROS) leading to oxidative stress and consequent cell damage [14,15]. Oxidants such as Cu, Fe and Ti are redox-active transition metals that may cause oxidation damage to lipids, protein and DNA metabolism. These elements catalyzes formation of reactive oxygen species through the Fenton and Haber-weiss reaction [16,17]. These oxidants have been shown to increase formation of atherosclerotic lesions in animal models and epidemiological data suggest that a direct relationship exists between the intake of oxidant and the risk of coronary artery disease [18].

Titanium ingestion could initiate some series of events that result in oxidative stress and might enhance the adverse conditions and further damage in normal situation. Because the possible link among titanium intake, oxidative stress and dyslipidemic profile, it would be of interest to examine the effects of titanium intake on lipid profile in normal condition. We have already been shown that titanium increased *in vitro* lipoprotein oxidation [19], which is the key step in the progression of atherosclerosis. Whether this is likely to occur *in vivo* and if so, under what condition needs more investigation [15,16]. We also showed that lipoprotein lipase activity [20] and liver contents of lipid fractions are changed following titanium treatment [20]. The present study was carried out to show whether

Table 1. The effect of acute dose of titanium (2.5 mg/kg for 10 days) on plasma lipid-related parameters

Parameter	Control group	Treated group	% Change
Cholesterol (mg/dl)	71 ±11.1	132 ± 39.5	+86.6*
Triglyceride (mg/dl	71.5±15.45	61.25±9	-14.3*
Free Fatty Acid (μmole/lit)	139.75±42	128.25±13.3	-8*
HDLC (mg/dl)	27.5±11.9	31.75±10.9	+15.5
LDLC (mg/dl)	29.5±20	88.5+12.7	+200*
VLDL (mg/dl)	14.25±2.22	12.25±1.71	-14*

Each value is the mean ± SD of five separate experiments performed in duplicate. Percent changes are shown in parenthesis. Stars indicate that the values are significantly different from controls. *Statistically significant ($p < 0.005$)

Table 2. The effect of chronic dose of titanium (0.75 mg/kg for 30 days) on plasma lipid-related parameters

Plasma	Control group	Treated group	% Change
Cholesterol (mg/dl)	76 ±12	127.5±10	+67.2*
Triglyceride (mg/dl	72.5±16.6	60±12.9	-17.2*
Free Fatty Acid (μmole/lit)	137.25±11.9	125±16.7	-8.9*
HDLC (mg/dl)	31.25±8.5	36.25±11.93	+16
LDLC (mg/dl)	30.5±14	78.5+16.25	+155.7*
VLDL (mg/dl)	14.5±1.73	12±2.6	-17.2*

Values are the mean ± SD of five separate experiments performed in duplicate. Percent changes of each parameter are also indicated. *Statistically significant ($p < 0.005$)

Table 3. The effect of chronic dose of titanium (0.75 mg/kg for 60 days) on rat plasma lipid and lipoprotein levels

Plasma	Control group	Treated group	% Change
Cholesterol (mg/dl)	81.25±13	136.25±7.5	+67.7*
Triglyceride (mg/dl)	77.25±11.62	50.5±12.93	-34.6*
Free Fatty Acid (μmole/lit)	136.25±20	119.25±14.9	-12.5*
HDLC (mg/dl)	24.75±8.8	28±4.5	+13.1
LDLC (mg/dl)	41.25±20.55	98.25+16.1	+138*
VLDL (mg/dl)	15.25±2.1	10±2.58	-34.4*

Values are the mean ± SD of five separate experiments performed in duplicate. Percent changes in each parameter are also indicated. *Statistically significant ($p < 0.005$)

titanium intake could induce dyslipidemic profile at *in vivo* condition.

MATERIALS AND METHODS

Male Wistar rats (200-250 g) were used for this study. They were kept under standard conditions having free access to food and water but were fasted the night before experiment. The experimental animals received daily intraperitoneal dose of 2.5 mg/kg body weight of titanium for a period of 10 days (acute dose) and 0.75 mg/kg body weight of titanium for a period of 30 and 60 days (chronic dose). In the day of experiment, animals were killed, bloods were collected and centrifuged at 2000-2500×g and their plasma was separated from blood cells. Samples were used directly or kept refrigerated for the analysis. Free fatty acids were measured by the method of Felix [21] and plasma protein by the method reported by Lowry *et al.* [22]. Plasma cholesterol and triglyceride levels were determined by the enzymatic methods using commercial laboratory kits purchased from Ziest Chimie (Tehran, Iran). HDL-C was measured in the supernatant after the precipitation of the Apo-B containing lipoproteins (LDL and VLDL) using polyanions in the presence of a divalent cation [23]. LDL-cholesterol was determined by calculation using the Freidewald equation [24].

RESULTS

Plasma levels of lipid related parameters were changed in animals treated with different doses of titanium (Tables 1-3). As indicated in Table 1, cholesterol and LDL-C increased significantly in animals treated with acute dose of titanium in short time whereas triglyceride and free fatty acids were decreased in this condition. Changes in lipid fractions in animals chronically treated with titanium for 30 and 60 days are shown in Tables 2 and 3 respectively. It can be seen that the pattern of changes is similar to acutely-treated animals.

DISCUSSION

The present results demonstrated that titanium interferes with lipid metabolism .This interference might be initiated by the changes in the activity of lipoprotein lipase, a key enzyme that plays an important role in the metabolism, transport and tissue uptake of lipid fractions. Our previous study showed that titanium reduced the activity of this enzyme by 19.6-36.1 percent ($p < 0.005$) [20]. The exact mechanism by which titanium inhibits lipoprotein lipase activity is not known exactly, however the activity of this enzyme depends on the presence of free SH groups [25]. It is probable that titanium by interacting with some essential SH groups in the active site of the enzyme reduces enzyme activity.

The reduction in the plasma levels of free fatty acids could be attributed to either the lowered activity of lipoprotein lipase in the presence of titanium or the inhibition of adenylate cyclase system leading to the reduction in intracellular levels of cAMP and the

inactivation of HSL [26]. Although the concentrations of different lipid and lipoprotein fractions are changed following the administration of titanium, the main clinically-important consequence is the significant increase in LDL levels of titanium treated animals. This effect of titanium should be considered seriously. It is well documented that there is a relationship between lipoprotein levels and the incidence of cardiovascular disease [27]. There are many reports indicating that the progression of atherosclerosis is well correlated with high plasma LDL/HDL ratio [27-29]. So, our results that titanium elevated the ratio of LDL/HDL should be considered seriously in patients taking this drug for a long period of time.

Our results also showed that the liver content of triglycerides decreased following the administration of titanium. Triglycerides are synthesized from the esterification of glycerol phosphate and acyl CoA. Glycerol phosphate in cells is partly maintained by glycolytic reactions, and titanium probably inhibits glycolytic enzymes. Therefore, the intracellular level of glycerol phosphate is limited. Titanium, on the other hand, inhibits lipoprotein lipase and lowers the plasma levels of free fatty acids. It is probable that the limitation of triglyceride synthesis may result in lower levels of liver triglycerides. Substrates that could not reach the triglyceride synthesis pathways may contribute to phospholipid synthesis. Our results showed that titanium increased phospholipid contents of liver.

Considering our data, it is concluded that the systemic changes in plasma lipid parameters, at the cellular level, may induce changes in cell membrane and metabolism and this could be the start of metabolic disorders in patients who are using titanium for a long period of time.

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