Iranian Journal of Pharmaceutical Research (2008), 7 (3): 179-183
Received: January 2008
Accepted: May 2008

Original Article

Changes in Liver Contents of Lipid Fractions Following Titanium Exposure

Alireza Ani, Mohsen Ani*, Ali Asghar Moshtaghie and Hassan Ahmadvand

Department of Clinical Biochemisry, School of Pharmacy, Isfahan University of Medical Sciences, Isfahan, Iran.

Abstract

The potential to cause tissue damage by metal ions is the matter of widespread investigation. Titanium salts are widely used in industry for ceramic painting, in pharmacy for tablet coating and making chemical sunscreens and in medicine as photo catalysts with bactericidal activity. This may address the idea that the exposure to these salts could play a role in metabolic disorders. In this study the effect of Ti on liver contents of lipid fractions was investigated.

Male Wistar rats (200-250 g) were used for the experiments. Groups of animals were injected for 10 days with 2.5 mg/kg of titanium chloride, as acute dose and for 30 and 60 days with 0.75 mg/kg as chronic doses. At the end of the experimental period animals were anaesthetized, the abdomens were opened and the livers were perfused with appropriate buffer. Livers were then removed immediately and used fresh or kept frozen until the analysis. Livers were then homogenized and their contents of triglycerides and phospholipids were determined. Blood samples were also collected before killing to measure the lipid levels.

Titanium led to a significant increase in phospholipid content of the liver (about 66 %) whereas triglycerides decreased by about 25 to 30 percent in all treated animals. Titanium also reduced plasma free fatty acids and triglycerides significantly but cholesterol and LDL levels were increased in all treated animals. Lipoprotein lipase activity was also inhibited in titanium treated animals.

In Conclusion This study is significant because it shows that chronic inhalation or exposure to titanium at workplaces is associated with changes in liver lipid metabolism. Plasma lipid-related parameters were also affected. Although less information is available concerning the mechanism of toxicity but the induction of reactive oxygen species production may be responsible for this effect.

Keywords: Titanium; Phospholipids; Triglycerides; Liver.

Introduction

The environmental and health risk of exposure to some commonly used trace metals is under widespread investigation. Drugs and chemical agents that contain any metallic substance may also undergo in vivo degradation which could then affect the intracellular metabolism and also the biological response of cells and tissues. The change in biological responses vary with the metal itself, the dose and the duration of the exposure.

One of these elements is titanium which is

* Corresponding author:
E-mail: ani@pharm.mui.ac.ir

widely used in industry, pharmacy and medicine. In pharmacy, titanium salts are used for tablet coating and in sunscreen preparations (1, 2). Titanium salts are also used in medicine as photo-catalysts with bactericidal activity (3). Ceramic painting is one of its applications in industry. Its use in surgery as dental implants, screws, plates and prosthese is also getting ubiquitous. Some plants and foods such as black pepper, cloves, thyme, lettuce, chicken and margarine have high contents of titanium (4). Taking titanium for a long period of time may accumulate in skin, lung, liver and heart (5). The widely usage of this element beside its toxic potential, justify any investigation about the biological and biochemical changes, induced by titanium salts. However, although it has been made clear that titanium could affect many aspects of cell metabolism, the exact mechanism of its effect is not very well characterized.

It has been reported that following absorption and entrance to blood circulation titanium binds strongly to transferring molecules and is carried to the cells and tissues (6). Titanium may then exert its effect through some intracellular signaling pathways leading to expression of certain protein and biomolecules (7). Various studies also indicate its potential to activate free radicals and also the role of reactive oxygen species in the process of toxification of this element (8).

In the course of in vivo search for the toxicity screening of chemicals on liver function, different aspects of metabolic reactions have been chosen to examine their adverse effects. Liver is actively involved in the manufacturing of many blood biomolecules, therefore its metabolism and functions are susceptible to alteration as a consequence of interaction with foreign particles.

Due to the importance of lipid metabolism in liver, this study describes the interaction of titanium with the levels of triglycerides and phospholipids.

Although the effect of titanium on the alteration of lipids in isolated cells has recently been reported (9) but searching the literature shows very little information about the in vivo effect of titanium on lipid metabolism especially in liver.

Experimental

Male Wistar rats were used for the experiments. They were kept under standard conditions having free access to food and water and their weight were 200-250 g at the time of experiments. Different groups of rats were chosen, 5 animals in each group. To study the acute effects of titanium in short term, a group of animals was injected for 10 days with 2.5 mg/kg of titanium chloride intraperitoneally. In long term study animals were injected with 0.75 mg/kg of titanium chloride for 30 and 60 days. Another group of animal was treated with normal saline and considered as control. At the end of the experimental period, animals were anaesthetized, their abdomens were opened and the livers were first perfused with appropriate buffer. Livers were then removed immediately and used fresh or kept frozen until analysis. Livers then were homogenized using Heidolph homogenizer (Model RZR3, type 50113) and their contents of phospholipids and triglycerides were determined. To do this, first total lipids of each liver was separated by the method of Norman (10) using a mixture of chloroform/ methanol (2/1: v/v) which then was washed with calcium chlorides solution. The upper aqueous layer was discarded. The lower chloroform layer contained most of lipid fractions of the liver which then were used for the separation of triglycerides and phospholipids. Norman method for separation of lipids is preferred because washing with calcium chloride avoids removal of some lipids that may be excluded if water were used for washing. Phospholipids were separated by the method of Rose *et all.* (11), which is based on the surface absorption of phospholipids by activated silisic acid and then removing them by appropriate solvents, which were then measured by phosphomolibdate method. The remaining triglyceride in the solution was measured by enzymatic methods using commercial kits based on lipase hydrolysis of triglyceride. For some preliminary experiments blood samples were also collected before killing of animals and their plasma were separated to analyze the lipid levels and also to

Table 1. The effect of acute dose of titanium (2.5 mg/kg) on liver lipid contents in animal treated for 10 days. Figures are Mean±SEM of 5 experiments performed in duplicate. Percent changes are also indicated in the table and stars show that the changes are significant.

	Control	Treated	Changes (%)
Phospholipids (mg/g)	0.21±0.05	0.34±0.08	+62%*
Triglyceride (mg/g)	0.31±0.08	0.24±0.06	-25%*

measure lipoprotein lipase activity.

Results

Preliminary experiments revealed that titanium affected plasma lipid parameters, the result of which is going to be published elsewhere. Thus plasma cholesterol and LDL were increased whereas free fatty acids and triglycerides decreased in all treated animals. Titanium could also affect plasma lipoprotein lipase activity and this effect showed to be inhibitory.

Results regarding lipid changes in the liver are shown in Tables 1 and 2. Table 1 shows the effect of acute dose of titanium on triglycerides and phospholipid contents of liver. As shown, phospholipids were increased by 62% in short term treated animals which was significantly different from controls. Triglyceride contents of the liver decreased by 25% under this condition.

Chronic effects of titanium on liver lipids are shown in Table 2. Again phospholipids were increased and triglycerides decreased in chronically treated animals.

After 30 days of treatment, 52 percent increase in liver phospholipids and 30 percent decrease in triglyceride were observed whereas after 60 days, phospholipids increased by 62 percent and triglycerides decreased by 25 percent.

Discussion

The biochemical side effects of titanium should be considered seriously due to the growing application of its products in medicine and industry. We have studied some biochemical properties of this element and the binding behavior of titanium and its potential to oxidize some lipoproteins have already been published (12, 13).

Reports also indicate that some aspects of lipid metabolism is affected in workers of titanium magnesium producers (14). There is no need to emphasize that any change in lipid metabolism and related parameters may affect whole body metabolism leading to serious consequences. Although it is not exactly known how titanium exerts its toxic effects on cells and tissues but there are suggestions that it may interfere with intracellular signaling pathways (7, 15), including cell cycle regulatory pathway, apoptosis pathway, the chemokine pathway and complement cascade as well as G-protein-coupled receptors (16). Titanium has also been suggested to play a role in the production of reactive oxygen species (ROS) in affected cells and tissues (17, 18). This property could pose a risk to many biological targets such as liver, brain and lung that are sensitive to oxidative stress damage (19). There is a bulk of reports indicating that reactive oxygen species interfere with lipid metabolism (20, 21) the out-come of which is changes in lipid storage and/or consumption. Liver is an important site for synthesis, sorting, packaging and secreting lipids. Putting together it is concluded that the production of reactive oxygen species, induced by titanium may contribute to the changes seen

Table 2. Changes in phospholipids and triglyceride contents of liver following a chronic dose (0.75 mg/kg) of titanium for 30 and 60 days. Figures are Mean±SEM of five experiments performed in duplicate. Percent changes are indicated in brackets and starred figures are significantly different from control animals.

	Control	Treated	
		30 days	60 days
Phospholipids (mg/g)	0.23±0.05	0.35±0.07 (+52%)*	0.38±0.08 (+66)*
Triglycerides (mg/g)	0.30±0.10	0.21±0.09 (-30%)*	0.25±0.06 (-17%)*

in lipid contents of the liver.

The reduction in triglyceride level seen in this study may also be attributed to the unavailability of free fatty acids for triglyceride production in the liver. We showed that titanium inhibited plasma lipoprotein lipase activity and reduced free fatty acid concentrations (unpublished data). On the other hand phospholipid contents of liver in titanium treated animals increased significantly.

It is well known that the synthesis of triglycerides in the liver is achieved by transfer of an acyl group to a diglyceride molecule which is the product of phosphatidate phospho hydrolase enzyme. When fatty acid is limited, diglyceride molecules so produced are diverted to phospholipid synthesis. This explains why the reduction in triglyceride levels is accompanied by the elevation of phospholipid contents of the liver.

In brief it may be suggested that titanium by interfering with cAMP-dependent signaling system (15) and decrease in lipolytic pathways leads to a reduction in free fatty acid supply so that lipid storage in the liver is affected.

Apart from titanium effect on lipid metabolism and liver function, many other interactions have been reported. For example titanium is shown to affect DNA and gene expression (22). Induced changes in gene expression are associated with a broad range of cell functional activities. Titanium is also reported to cause cell hyperplasia (23) and alter cell metabolic behavior even at sub toxic concentrations (24).

In view of this experimental results it can be concluded that workers in industry as well as all subjects who are exposed to titanium salt products for a long period of time should be checked occasionally, and even any minor changes in the levels of biochemical parameters and abnormality in liver and kidney function test should be considered serious to prevent the biohazards of this toxic elements.

References

(1) Wang K. The use of titanium for medical application in the USA. *Mater. Sci. Eng*. (1996) 213: 134-7

(2) Lademann J, Weigmann H, Rickmeyer C, Barthelmes H, Schaefer H, Mueller G and Sterry W. Penetration of titanium dioxide micro particles in sunscreen formulation in to the horny layer and the follicular orifice. *Skin. Pharmacol. Appl. Skin. Physiol*. (1999) 12: 247-56

(3) Maness P, Smolinski SH, Blake DM, Huang Z, Wolfrum EJ and Jacoby WA. Bactericidal activity of photocatalytic TiO_2 reaction: toward an understanding of its killing mechanism. *Appl. Environ. Microbiol*. (1990) 65: 4094-8

(4) Caruso F and Fossi M. Antitumor titanium compounds. *Mini. Rev. Med. Chem*. (2004) 4: 49-60

(5) Tsuji JS, Maynard AD, Howard PC, James JT, Lam CW, Warheit DB and Santamaria AB. Research strategies for safety evaluation of nanomaterials, part IV: risk assessment of nanoparticles. *Toxicol. Sci*. (2006) 89: 42-50

(6) Qian ZM, Li H, Sun H and Ho K. Targeted drug delivery via the transferrin receptor mediated endocytosis pathway. *Pharmacol. Rev*. (2002) 54: 561-86

(7) Nakashima Y, Sun DH, Trindade MC, Maloney WJ, Goodman SB, Schurman DJ and Smith RL. Signaling pathways for tumor necrosis factor-alpha and interleukin-6 expression in human macrophages exposed to titanium-alloy particulate debris in vitro. *J. Bone Joint Surg. Am*. (1999) 81: 603-15

(8) Long TC, Saleh N, Tilton RD, Lowry GV and Veronesi B. Titanium dioxide (P25) produces reactive oxygen species in immortalized brain microglia (BV2): implications for nanoparticle neurotoxicity. *Environ. Sci. Technol*. (2006) 40: 4346-52

(9) Schuster GS and Caughman GB. Alterations of cell lipids by metal salts. *J. Biomed. Mater. Res*. (2004) 70A: 347-353

(10) Norman SR. Preparation of lipid extracts. In: *Methods in Enzymology* J.M. Lowenstein (editor) Vol. 14, Academic Press, London (1969) 245-250

(11) Rose H, Vaughan M and Steinberg D. Utilization of fatty acids by rat liver slices as a function of medium concentration. *Am. J. Physiol*. (1964) 206: 345-350

(12) Moshtaghie AA, Ani M and Arabi MH. Specteroscopic studies on titanium ion binding to the apolactoferrin. *Iran Biomed. J. (*2006) 10: 93-98

(13) Ani M, Moshtaghie AA and Ahmadvand H. Comparative effects of copper, iron, vanadium and titanium on low density lipoprotein oxidation *in vitro*. *Iran Biomed. J*. (2007) 11: 113-8

(14) Sultanbekov ZK. Lipid peroxidation and antioxidant defense among workers of titanium magnesium production. *Med. Tr. Prom. Ekol*. (2004) 11: 12-6

(15) Blaine TA, Pollice PF, Rosier RN, Reynolds PR, Puzas JE and O'Keefe RJ. Modulation of the production of cytokines in titanium-stimulated human peripheral blood monocytes by pharmacological agents. The role of cAMP-mediated signaling mechanisms. *J. Bone Joint Surg. Am*. (1997) 79: 1519-28

(16) Chen HW, Su SF, Chien CT, Lin WH, Yu SL, Chou CC, Chen JJ and Yang PC. Titanium dioxide nanoparticles induce emphysema like injury in mice. *Faseb J*. (2006) 20: 2393-5

(17) Lee MC, Yoshino F, Shoji H, Takahashi S, Todoki K,

Shimada S and Kuse-Barouch K. Characterization by electron spin resonance spectroscopy of reactive oxygen species generated by titanium dioxide and hydrogen peroxide. *J. Dent. Res*. (2005) 84: 178-82

(18) Long TC, Tajuba J, Sama P, Saleh N, Swartz C, Parker J, Hester S, Lowry GV and Veronesi B. Nanosize titanium dioxide stimulates reactive oxygen species in brain microglia and damages neurons *in vitro*. *Environ. Health Perspect*. (2007) 115: 1631-7

(19) Gurr JR, Wang AS, Chen CH and Jan KY. Ultrafine titanium dioxide particles in the absence of photoactivation can induce oxidative damage to human bronchial epithelial cells. *Toxicology* (2005) 213: 66-73

(20) Gago-Dominguez M, Castelao JE, Pike MC, Sevanian A and Haile RW. Role of lipid peroxidation in the epidemiology and prevention of breast cancer. *Cancer Epidemiol. Biomarkers. Prev*. (2005) 14: 2829-39

(21) Wood LG, Gibson PG and Garg ML. Biomarkers of lipid peroxidation, airway inflammation and asthma. *Eur. Respir. J*. (2003) 21: 177-86

(22) Carinci F, Volinia S, Pezzetti F, Francioso F, Tosi L and Piattelli A. Titanium-cell interaction; analysis of gene expression profiling. *J. Biomed. Mater. Res. B. Appl. Biomater*. (2003) 66: 341-6

(23) Ahn MH, Kang CM, Park CS, Park SJ, Rhim T, Yoon PO, Chang HS, Kim SH, Kyono H and Kim KC. Titanium dioxide particle-induced goblet cell hyperplasia: association with mast cells and IL-13. *Respir. Res*. (2005) 13: 34

(24) Sun ZL, Wataha JC and Hanks CT. Effects of metal ions on osteoblast-like cell metabolism and differentiation. *J. Biomed. Mater. Res*. (1997) 34: 29-37

This article is available online at http://www.ijpr-online.com