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Comparative *in vitro* Study of the Intestinal Absorption of Titanium and Iron in Rats

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Abstract: Rat Everted Gut Sac (EGS) model was employed to study the intestinal uptake of titanium and iron. Incubation of freshly prepared rat EGS in Earle's medium pH = 7.4 containing titanium showed that the absorption of titanium as well as iron was a dose dependent process. Ascorbic acid enhanced the absorption of both metal ions, while NaF (1 mM) as an inhibitor of glycolytic energy supply, decreased their absorption. The Na^+-K^+ ATPase inhibitor, ouabain (1 mM) reduced intestinal absorption of Titanium. This suggests that titanium uptake is an active transport process as is iron uptake. Iron absorption was reduced approximate by 17% when titanium was presented to incubation medium EGS whereas, the absorption of titanium was decreased by 35% when iron was added to the reaction mixture.

Key words: Titanium, iron, metabolism, everted gut sac

INTRODUCTION

Titanium (Ti) is a relatively common element in the earth's crust, yet it is often thought to be inert in biology and environment (Wells, 1984). The consumption of this metal and it's biomedical applications has recently increased. The specific properties of resistance to corrosion and inertness allow many metallurgical applications of Ti in daily life (Kohn, 1998; Yang *et al*., 2009; Elias *et al*., 2008).

Titanium alloys are now the most attractive metallic materials for biomedical applications. They are used for medical devices such as hip joints, bone screws, knee joints, bone plates, dental implants, surgical devices and pacemaker cases due to their total resistance to attack by body fluids, high strength and low modulus (Yang *et al*., 2009; Suwalsky *et al*., 2005).

Some titanium compounds have been shown to exhibit high antitumor activates against a wide range of murine and human tumors with less toxic side effects than platinum anti-tumor agents (Tshuva and Ashenhurst, 2009; Caruso and Rossi, 2004; Melendez, 2002; Kopf-Maier and Kopf, 1994; Desoize, 2004).

Titanium (IV) citrate has also been tested against tumors in rats. In a research rats with Jensen's sarcoma were divided into two groups, one of which was treated with injections of Ti(IV) citrate. Three-week survival rates were 88% for titanium-treated group and 39% for control group (Collins *et al*., 2005).

Titanium occurs widely in the natural environment and is detectable in many kinds of foods (Yang *et al*., 2009). Its content in black pepper, cloves, thyme, lettuce, pork, chicken and margarine is high (Suwalsky *et al*., 2005; Tshuva and Ashenhurst, 2009). It has also replaced lead in paints; inhalation of TiO_2 containing particles contributes to titanium accumulation in skin, lung, spleen, liver and heart (Suwalsky *et al*., 2005). It has also been reported that approximately one third of patients with titanium alloy spinal implants exhibit abnormal serum concentrations and that titanium may travel to distant organs after its dissolution from spinal implants (Suwalsky *et al*., 2005; Silwood and Grootveld, 2005).

In spite of the widespread biomedical use of titanium compounds and titanium alloys, the metabolism of Ti has not been fully investigated even in animal models. There is very limited data which exists on the intestinal Titanium uptake. The objectives of the present study was to investigate and compare titanium and iron absorption by the rat everted gut sac techniques to study the effects of various factors on this process.

MATERIALS AND METHODS

Materials: All chemicals used in this project were of analytical grade and purchased from Sigma Chemical Co and Merck. Laboratory glassware including covettes were routinely soaked overnight in 10% HNO_3 and washed three times with distilled and deionized water before use to minimize metal ions contamination. Titanium concentration in distilled and deionized water was determined using flameless-atomic absorption spectrophotometry.

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Everted gut sacs preparation and incubation: Experiments were carried out on male wistar rats kept in faculty animal house at standard conditions. Rats were fed on basal diet and water until their weights reached between 200-250 g. Animals were starved 24 h prior to the experiment and they were killed by cervical dislocation. Their small intestines were quickly excised and removed, washed with NaCl solution (0.9% w/v) at room temperature, blotted, dried and weighted. The intestines were cut into small pieces and proximal duodenum, jejunum segments were prepared. The segments were gently everted over a glass rod (2.5 mm diameter) and one end of pieces was closed using braided silk sutures (Arellano *et al*., 2004; Wilson and Wiseman, 1954). The everted gut sacs were filled up with Earl's medium pH = 7.4 and suspended in Earle's medium either with or without iron and/or titanium. The incubation mixture was capped and gassed with O_2/CO_2 = 95/5 in water bath shaker at 37°C. At appropriate time intervals sacs were removed, washed three times in saline and blotted dry. The sacs were cut open and the serosal fluid was drained into small tubes for subsequent analysis. Titanium concentration was measured from inside the sacs using atomic absorption spectrophotometry (Perkin elmer series 300). Protein concentration was determined by Bradford method (Bradford, 1976).

Preparation of titanium and/or iron citrate complex: Titanium (IV)citrate was prepared by mixing titanium (III)chloride with a 1.2 fold excess of sodium citrate at pH 3; exposure to air caused quantitative oxidation of titanium (III) citrate to colorless titanium (IV) citrate (Messori *et al*., 1999). Separate standard solutions of iron chloride were prepared in distilled and deionized water and mixed with equal volumes of citric acid 1:1.2. The solutions were adjusted to pH = 7.4 with 1 M NaOH and to get to the final concentration. The solution was always prepared freshly on the day of the experiment. The final concentration of Ti (IV) and Fe (III) in each solutions was determined by the above mentioned method.

RESULTS AND DISCUSSION

Preliminary experiments have been carried out to establish optimum conditions necessary for the iron uptake study by EGS (Moshtaghie *et al*., 1997, 2006a,b). First, the level of iron uptake was investigated. The results presented in Fig. 1 and 2 show that maximum Fe (III) uptake by EGS is up to 300 μg dL^{-1} (Fig. 1) and the level of Ti (IV) uptake is approximately 200-300 μg dL^{-1} (Fig. 2). When NaF was used to inhibit the glycolytic energy supply, 13 and 9% decrease in iron and titanium absorption was observed, respectively (Fig. 3, 4). Ouabain, a specific inhibitor of the

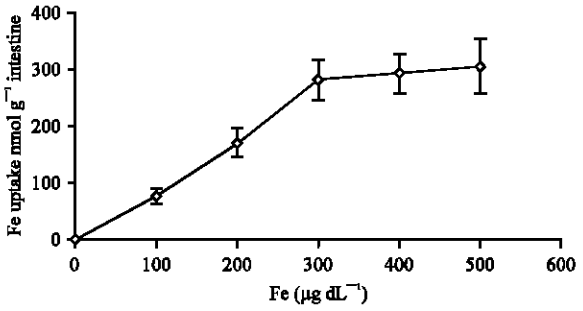

Fig. 1: Iron uptake by rat EGS, the effect of concentration. Each value represents the mean of the "n" experiments

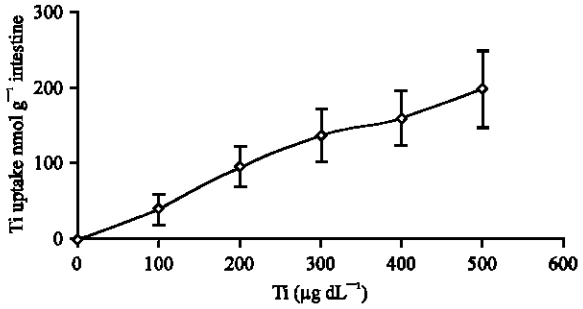

Fig. 2: Titanium uptake by rat EGS, the effect of concentration. Each value represents the mean of the "n" experiments

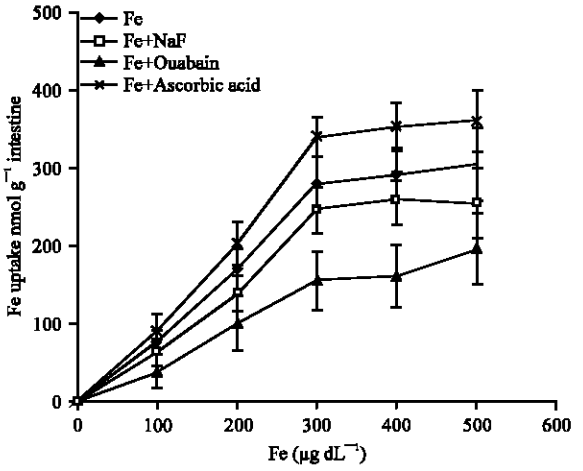

Fig. 3: Influence of NaF (1 mM), Ouabain (1 mM) and ascorbic acid (500 mg L^{-1}) on Iron uptake by EGS. Each value represents the mean of the "n" experiments

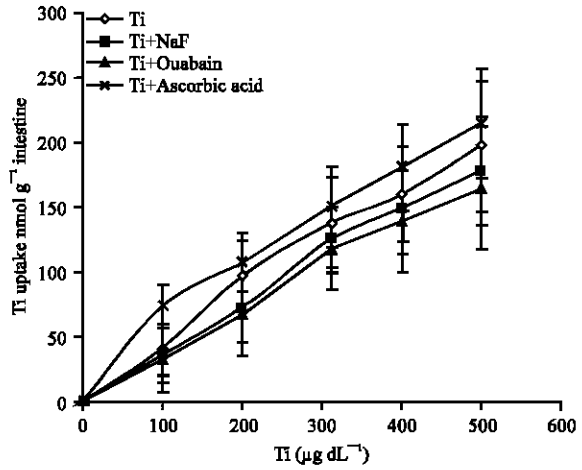

Fig. 4: Influence of NaF (1 mM), Ouabain (1 mM) and ascorbic acid (500 mg L^{-1}) on titanium uptake by EGS. Each value represents the mean of the "n" experiments

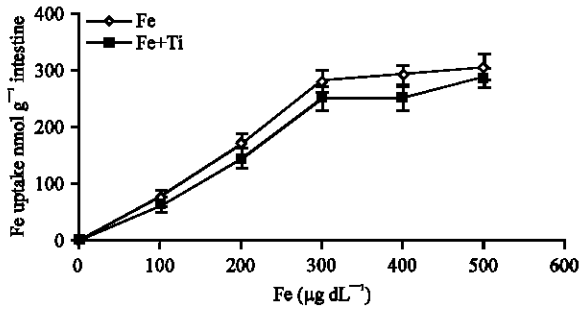

Fig. 5: Effect of titanium on iron absorption by EGS. Each value represents the mean of the "n" experiments

membrane Na^+/K^+ ATPase, also caused 33 and 24% reduction in iron and titanium uptake, respectively (Fig. 3, 4).

Additions of ascorbic acid to the medium accelerated iron absorption through small intestine (Mackenzie and Garrick, 2005). In this study presence of ascorbic acid (500 mg L^{-1}) in media caused 21 and 15% increase in iron and titanium absorption, respectively. Finally, the intestinal interaction between iron and titanium was studied. Iron absorption was reduced approximately 13% when Ti (250 µg dL^{-1}) was presented to the incubation media (Fig. 5); whereas the addition of iron caused 35% decrease in titanium absorption (Fig. 6).

To the best of our knowledge, this is the first study to investigate titanium uptake through intestine using rat everted gut sac. Previous studies in this laboratory have shown that titanium interference with iron binding by

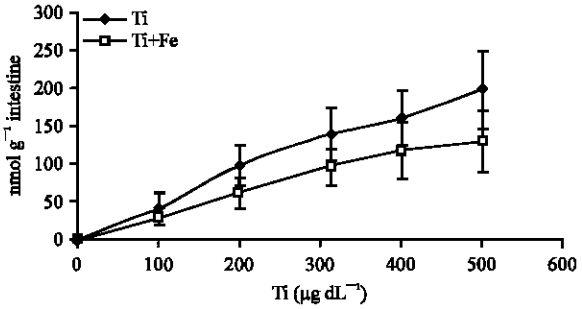

Fig. 6: Effect of iron on titanium absorption by EGS. Titanium concentration Each value represents the mean of the "n" experiments

lactoferrin (Moshtaghie *et al*., 2006a) and transferring (not reported). Since transferrin has also been reported to play an important role in iron and cadmium uptake by intestine (Moshtaghie *et al*., 1996), it is obvious that there should also be a possibility of the interaction between Ti and Fe at absorption site in intestine. Metal absorption and its transfer across the intestine is complex and involve an integration of biochemical and biophysical processes. The data which has been presented in this study shows that titanium absorption by EGS depends on some factors including concentration, metabolical energy and presence of other metal ions such as Fe^{3+}.

Compared with Iron absorption, intestinal uptake of Ti (IV) is slightly different and is biphasic. This difference in behavior is related to the toxicity of titanium and its chemical properties. In the case of iron the first phase is saturable and has the characteristics possibility of a carrier mediated process. The second phase is not satureable and may be independent of carrier mediated protein. Suwalsky *et al*. (2005) have been reported that titanium citrate induced shape changes in erythrocytes (Suwalsky *et al*., 2005). This effect can be extended to other cells and affect their functions. We can conclude that the presence of titanium ions in a high dose (second phase) may perturbate the enterocyte membrane and interfere with intestinal uptake.

The data presented in this study shows that titanium interferes with iron absorption and that iron causes decrease in titanium uptake. The results of this study are in line with those reported in the literature on the effect of iron on the absorption of some elements (Arnich *et al*., 2004). They also agree with the studies of the addition of iron, inhibiting manganese and showing a trend to increase in nickel absorption (Arnich *et al*., 2004; Tallkvist and Tjalve, 1994). It has been reported that high dietary of iron depresses some metal ions absorption such as manganese (Moshtaghie *et al*., 2006b; Finely, 1999)

and the addition of iron to an intestinal perfusate decreases manganese absorption (Thompson *et al*., 2006). According to earlier studies, non heme iron is transported across the enterocyte mainly by the carrier DMT1 (Divalent Metal Transporter 1) that facilitates the cellular absorption of divalent metal ions (Courville *et al*., 2006). Furthermore, the data from some studies have indicated that DMT1 is a nonspecific metal transporter which can transport not only Fe but probably other metal as well (Park *et al*., 2002).

Therefore, we can conclude that Ti and Fe might share a common transport mechanism. However, more investigations need to be carried out to elucidate the exact mechanisms involved.

In the present study the effect of ascorbate as a ligand and iron reducer agent in intestine was studied. The results showed that the presence of ascorbate was less effective on titanium uptake than on iron uptake which can be related to the different chemical properties of titanium and iron.

Previous findings have shown that some ions transportation across intestine membrane require energy which is provided by ATPase located in the membrane following the release of energy from ATP molecules (Richards *et al*., 1987; Trischitta *et al*., 2004).

Our data show that transportation of Titanium and iron requires energy provided by metabolical energy and ATPase system. This might be needed for the active transport process.

Addition of ouabain (500 mg L^{-1}) has decreasing effects on both titanium and iron absorption by intestine. It could be assumed that titanium uptake is an active transport process as is iron uptake. We think at this point more investigation should be done to elucidate deeply the exact mechanism by which titanium absorption is performed in the intestine.

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