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Toxicokinetics and tissue distribution of titanium in ionic form after intravenous and oral administration

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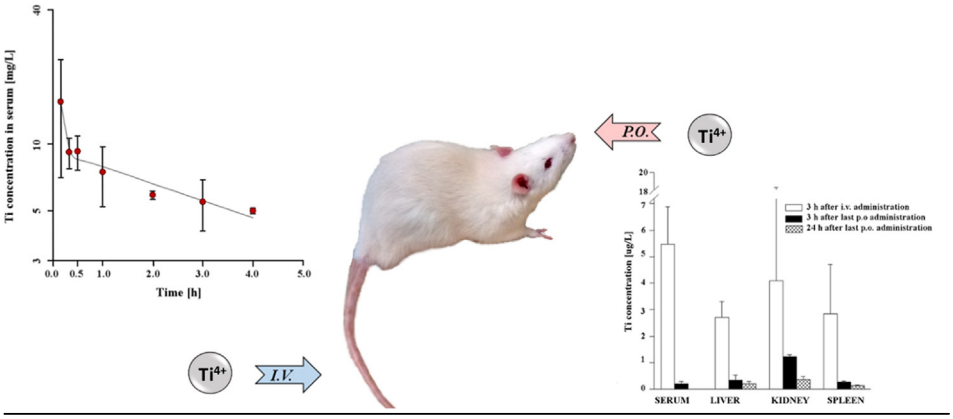
HIGHLIGHTS

- Investigation of the distribution pattern of ionic titanium in rats.
- Single intravenous or 30-days oral administration of 6 mg Ti/*b.w.* in ionic form.
- Kidney identified as the main target tissue after both routes of administration.
- Increase of Ti level in most organs after a month of oral exposure.

GRAPHICAL ABSTRACT



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ABSTRACT

Titanium is widely used both in food and cosmetics, as well as in surgery and industry. Contrary to most studies, the present work focused on the determination of the toxicokinetic parameters of titanium in ionic form, as well as on its tissue biodistribution in rats. The animals were administered either a single intravenous dose of 6 mg Ti/kg *b.w.*, or received the same dose orally every day for 30 days. The concentration of titanium in the serum and organs was measured by a graphite furnace atomic absorption spectrometry. Metal rapidly distributed from the circulation to the investigated organs after both routes of administration, and kidney was identified as the main target tissue, followed by liver and spleen. One month of oral exposure to Ti led to the increase of its concentration in liver, kidneys, spleen, and heart. In the intravenous study, both the highest area under concentration-time curves and the longest elimination half-life time were recorded in the kidney followed by serum, spleen and liver. The present study contributes to the knowledge of the toxicokinetics of titanium in ionic form, which may be especially useful when assessing the health risks of long-term exposure to titanium alloy implants in patients.

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1. Introduction

Until recently, higher levels of titanium in humans have not been seen as a real concern, because this metal was considered almost completely biologically inert. However, an increasing number of studies have addressed the toxicological aspects of Ti. Titanium is used extensively in medicine, food, cosmetic and other industries (Weir et al., 2012). Therefore, contrary to popular belief, Ti is widely distributed in the environment, and can easily enter the body through a variety of routes: oral, dermal, or directly from surgical implants made of titanium alloys. In the food industry this element is used as an additive in the form of titanium dioxide, denoted with E171, which serves as a white color enhancer in products like chewing gums and candies, or as a taste enhancer (Lomer et al., 2000; Weir et al., 2012). TiO_2 can also be found in a wide variety of personal care products and cosmetics such as creams, toothpaste, deodorants, and sunscreens (Liu et al., 2013; Schwab et al., 2012; Weir et al., 2012).

Today the mechanism of action of titanium on the human body is not yet fully understood, but it is known that metal can cause adverse health effects as a result of, for example, inhalation (Grassian et al., 2007; Huang et al., 2015; Ma-Hock et al., 2007; Wang et al., 2007; Warheit et al., 2007). Titanium alloys are of great value in orthopedic surgery due to their significant resistance to the attack of body fluids, strength, flexibility, biocompatibility, or lack of allergenicity (Niinomi, 2008). However, many scientific studies have shown that the components of biomaterials can be released into the body (Cundy et al., 2014; Frisken et al., 2002; Leopold et al., 2000; Matusiewicz, 2014; Nuevo-Ordóñez et al., 2011; Patton et al., 2008). This indicates that the implants undergo corrosion under unfavorable conditions in the internal environment of the organism. The signs of degradation can be observed both in the tissues adjacent to the implant (metallosis), and in the implanted biomaterial (Grosse et al., 2015; Shibli et al., 2005). Many factors such as relatively high, constant temperature, the presence of various ions, dissolved oxygen, and proteins, tribological conditions and an acidic environment, appearing at the site of the implanted medical devices, lead to degradation of the protective layer on the surface of implants (Bhola et al., 2011). As a result, particles or titanium ions are released from the biomaterial into the surrounding tissues. Studies on the ionic forms of titanium are necessary because, as has been demonstrated by Leopold et al. (2000), long-term use of an implant can result in the release of a considerable amount of metal ions. Another effect of corrosion is an increase of the Ti level in blood (Cundy et al., 2014; Lazennec et al., 2009; Leopold et al., 2000; Nuevo-Ordóñez et al., 2011; Patton et al., 2008) or even hair (Rodríguez de la Flor et al., 2013). At the beginning of the treatment it is small, but after a long time of implantation the concentration of metal can be up to 100 times higher (Leopold et al., 2000).

Publications that have appeared in recent times usually assess the exposure and health effects that titanium dioxide nanoparticles have on various organs (Cho et al., 2013; Elgrabli et al., 2015; Eydner et al., 2012; Fabian et al., 2008; Geraets et al., 2014; Wang et al., 2007; Wu et al., 2009). There is no information about the behavior of Ti in ionic form in the body. In contrast to most reports, the present study concerns the toxicokinetics of titanium in an animal model. Metal in ionic form was given to rats *via* the intravenous or oral route. The aim of this study was to determine the toxicokinetic parameters of ionic titanium and investigate the distribution pattern of titanium ions in rats. We would also like to compare the effects of intravenous and oral administration of metal on the organism as well as compare the results with the literature data on titanium in the form of nanoparticles. So far there is no information about the systematic research in this area. A closer study of this issue is needed because of the potential exposure of the human population to titanium compounds and possible toxic effects in the future.

2. Materials and methods

2.1. Chemicals and reagents

Deionized water (resistivity 18.2 MΩ·cm) obtained from the WG-HLP deionization system (Wigo, Poland) was used for the dilution of samples and reagents. Concentrated nitric acid (65%, Suprapur®, Merck, Germany) was used for digestion. Titanium calibration standards (20, 40, 60 and 80 μg/L) were prepared by diluting a 1 g/L titanium standard stock solution (Titrisol, Merck, Germany) with 1% HNO_3. The following chemical modifier solutions, purchased from Merck (Germany), were used: magnesium modifier (10.0 ± 0.2 g/L $Mg(NO_3)_2$ in 17% (v/v) HNO_3), and phosphate modifier (100 ± 2 g/L $NH_4H_2PO_4$ in H_2O). Titanium(IV) citrate was synthesized in Department of Inorganic Chemistry, Faculty of Chemistry, Jagiellonian University in Krakow, according to the method described by Deng et al. (2004). The certified reference material of the tissue was not commercially available, so the accuracy of the method was assessed by analysis of the CRM of serum (280 ± 42 μg Ti/L, UTAK Laboratories Inc., USA). A CRM of bovine liver (BCR-185R, Institute for Reference Materials and Measurements, Belgium) was used for the recovery studies. Argon (Linde, Poland) with a purity of 99.999% was used as the purge gas.

2.2. Animal experiments

Male albino Wistar rats, weighing between 150 and 250 g, were used for the experiments. The animals were housed and fed in a laboratory and kept at a constant temperature of 22 °C under standard conditions (12:12 h L:D cycle, standard pellet diet, tap water). All of the experiments were approved by the Local Ethics Committee of the Jagiellonian University in Krakow.

The animal experiments were divided into two parts in order to evaluate the patterns of ionic titanium distribution for different exposures. The rats were administered 6 mg Ti/kg body weight (*b. w.*) in the form of an aqueous solution of titanium(IV) citrate. The first group of animals (n = 21) received a single intravenous (*i.v.*) bolus injection to the tail vein. At 10, 20, 30 min and 1, 2, 3 and 4 h after the injection of Ti, the animals (n = 3 per time point) were sacrificed, and the liver, spleen, kidneys, and blood were collected. In a long-term study a solution of titanium salt was given to six rats by a nasogastric tube via the gastrointestinal tract (*p.o.*) daily for thirty days. At 3 h and 24 h after the last administration of Ti, the animals (n = 3 per time point) were euthanized, and the liver, spleen, kidneys, lungs, heart and blood were collected. Animals in the control group (n = 3) did not receive titanium salt.

2.3. Sample preparation

To obtain serum blood samples were allowed to stand at room temperature for 30 min to allow clotting and then centrifuged at 2500 rpm at 4 °C for 15 min. For elemental analysis samples were digested in a microwave digestion system Mars 5X (CEM, USA). About 0.3 g of tissue was transferred to a microwave vessel, 6 mL of HNO_3 was added and the samples were left for predigestion at room temperature for at least 12 h. In the case of serum, 5 mL of HNO_3 was added to 0.5 mL of the sample directly before digestion. The following three-step microwave program was used for the decomposition of samples: ramp to 160 °C in 4 min and hold for 4 min, ramp to 180 °C in 4 min and hold for 4 min and finally ramp to 200 °C in 4 min and hold for 7 min. After cooling to room temperature (30 min), the digests were diluted to 10 mL with