Titanium transport through the blood stream. An experimental study on rats

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Different metals are increasingly being used to manufacture implants, especially in the fields of dentistry and orthopedics. No metal or alloy is completely inert *in vivo*. The metal and the organic fluids interact releasing, for example, metallic products. Several hypotheses regarding the probable dissemination routes of titanium have been postulated, but its valence, the organic nature of its ligands and its potential toxicity have yet to be established. In a previous experimental study we demonstrated that i.p. injected titanium and zirconium oxides disseminate and deposit in organs such as liver and lung. The aim of this work was to study the eventual participation of blood cells in the transport mechanism of titanium employing the intraperitoneal injection of titanium oxide in rats as the experimental model. Twenty male Wistar rats, $\overline{x}$: 100 g body weight, were intraperitoneally injected with 16×10^3 mg/kg b.w. of TiO_2 in saline solution. Blood samples were taken by heart puncture at 3 and 6 months; blood smears were performed and stained with safranin evidencing monocytes containing titanium particles. The results obtained in this study would indicate that one of the ways in which titanium is disseminated is through the blood stream, via blood cells.

Introduction

Intraosseous titanium implants are used as a therapeutic alternative in rehabilitation both in orthopedics and in dentistry [1–3].

Experimental [4–9] and clinical [10–13] studies on the response of bone tissue have confirmed the biocompatibility of titanium.

The manufacturers of the different systems of implants strive to achieve an adequate design. Their choice of materials is aimed at guaranteeing minimum degradation, corrosion, dissolution, deformation and fracture among other properties [14–20].

Experimental studies have shown that osseointegration may be initially excellent but fail at a later date due to factors related to the implant itself or to the environment [5, 21–23]. Corrosion can be a cause of implant failure following a successful initial phase [24, 25].

An important property of titanium is that on exposure to air or liquids (H_2O) it rapidly develops a layer of oxide that reduces its reactivity [3, 26, 27]. In effect, it is this layer of oxide that interacts with the tissues. However, no metal or alloy is completely inert *in vivo*. The metal and the organic fluids interact releasing, for example, metallic products as a result of electrochemical processes [28].

Experimental and clinical studies have shown that once these products have been released they can accumulate locally or be systemically distributed [29–34].

In a previous study we found that titanium and zirconium oxides injected intraperitoneally in the rat disseminate and form deposits in target organs such as liver and lung 5 months post-administration [35].

Various studies have evaluated the concentration of metallic ions from implants in serum and blood cells [15, 28, 36–38]. Nickel, chromium and cobalt are transported bound to blood cells and/or proteins, in

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particular albumin [39, 40]. Aluminum is transported in serum bound to transferrin [41].

In the particular case of titanium, various dissemination mechanisms have been postulated [28, 33, 34, 42]. The aim of the present study was to study the eventual participation of blood cells in the transport mechanism of titanium employing the intraperitoneal injection of titanium oxide in rats as the experimental model.

Materials and methods

Thirty male Wistar rats, 100 g in body weight were employed. The guidelines of the National Institute of Health (NIH) for the use and care of laboratory animals were followed (NIH Publication No. 85-23, Rev. 1985).

A single intraperitoneal injection of a suspension of titanium oxide (TiO_2) (anatasa; Sigma Chemical Company, USA) ($n = 20$) was administered at a dose of 16×10^3 mg/kg body weight in 5 ml saline solution. The sizes of TiO_2 particles were about 1 μ, and they showed a sphere-like shape. A control group of animals ($n = 10$) was given an intraperitoneal injection of equivalent volumes of saline solution to evaluate the effect of the vehicle.

Blood samples (2 cm^3) were taken by intracardiac puncture (20G Terumo-USA needle) at 3 and 6 months. Blood smears were prepared and stained with safranin for identification of possibly phagocytosed material.

All animals were killed at 6 months by an overdose of ether. Systematic autopsies of all animals were performed. Samples of liver, spleen, kidney and lung were obtained, fixed in 10% neutral buffered formalin and embedded in paraffin. One set of sections was stained with hematoxylin and eosin and another set was prepared unstained. In all the cases, the sections were treated with saturated solutions of picric acid to remove formalin pigments. In the cases in which macrophages were loaded with metallic particles the sections were stained with PAS.

Samples of liver, spleen, kidney and lung were submitted to enzymatic digestion with trypsin. The sediment was analyzed crystallographically by X-ray diffraction to evaluate the presence of titanium.

Results

Control animals ($n = 10$) did not show alterations in body weight, behavior or general health throughout the experimental time.

At 10 days post-injection, seven animals in the experimental group evidenced significant ascites. An abdominal puncture was performed and a sample of a yellowish liquid (3 cm^3) was centrifuged at 1600 rpm for 5 min. Smears of the sediment were prepared and stained with hematoxylin–eosin. The presence of abundant peritoneal macrophages loaded with particles, neutrophils and lymphocytes were observed (Fig. 1). The EDX analysis performed to identify the material contained within the macrophages confirmed the presence of titanium (Fig. 2).

The analysis of the necropsies of the experimental animals ($n = 20$) revealed the presence of whitish deposits in the abdominal area. No macroscopic alterations were detected in liver, spleen or lung.

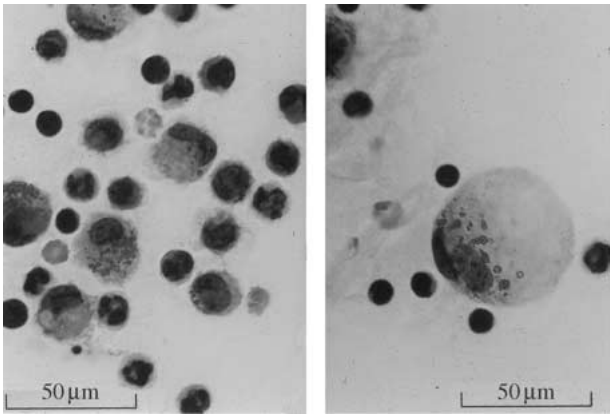

Figure 1 Smear of peritoneal liquid showing lymphocytes, neutrophils and material (TiO_2) within the cytoplasm of macrophages (H–E).

Histological studies

The organs of control animals failed to evidence alterations.

Experimental group. The histological analysis of the organs evaluated showed the same features as the samples examined in our previous study at 5 months post-injection of TiO_2 [35]. The presence of pigments in the organs could not be attributed to the presence of formalin pigments.

Liver. Macrophages loaded with particles, in particular in the sinusoid capillaries were observed. The liver surface failed to evidence macrophages (Fig. 3).

Lung. Foci of macrophages loaded with particles were detected (Fig. 4).

Spleen. The presence of material in the parenchyma was observed (Fig. 5).

Kidney. The tissue appeared normal.

In the organs analyzed, the macrophages loaded with particles showed an intense PAS reaction in liver and lung.

X-ray diffraction

The sediment of the enzymatic digestion of the tissues evidenced the presence of titanium oxide (anatasa).

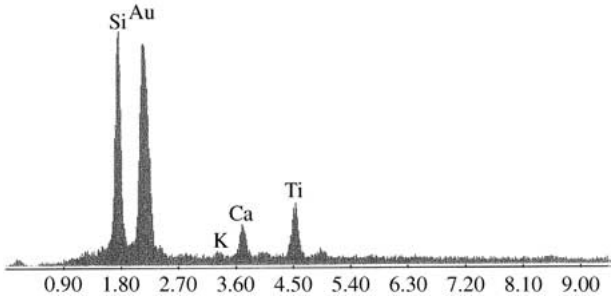

Figure 2 EDX analysis showing the presence of titanium in the particles analyzed.

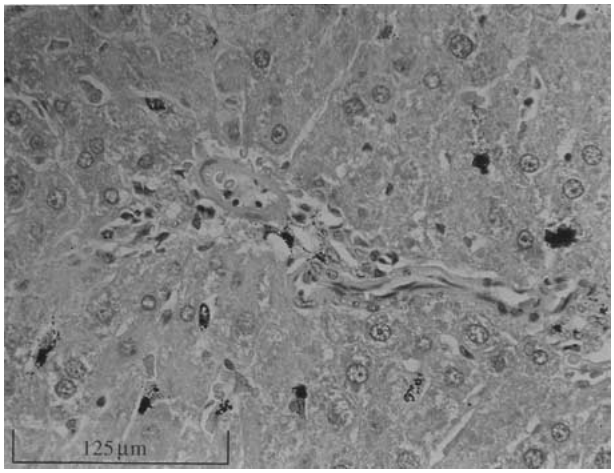

Figure 3 Liver. Note the presence of TiO_2 deposits (H–E).

Histological study of blood smears

Both at 3 and 6 months, cells of the phagocytic-mononuclear line loaded with particles were only present in the smears of the experimental group (Fig. 6). The intracytoplasmatic deposits showed different distribution patterns (Fig. 7).

Discussion

No metal or alloy is completely inert *in vivo*. Interactions between the metal and the bio-environment always occur [43]. This interaction leads to the slow but constant release of metallic ions. The chemically active metal ions may bind to the surrounding tissues but may also bind to proteins and be disseminated in the vascular and lymphatic systems to distant organs [31, 38]. The results of our study at 6 months post-injection of TiO_2 are in agreement with these findings.

Several studies have addressed the issue of systemic dissemination of metals to different organs [29, 38, 44]. In all these studies the presence of metallic ions in tissues was detected by special techniques such as atomic absorption spectrophotometry. Urban *et al*. [34] demonstrated the presence of metallic and plastic particles in organs such as liver, spleen and lymph nodes in post-mortem studies from patients with coxo-femoral prosthesis and knee replacements. In the present study the presence of titanium particles was confirmed in liver, spleen and lung at 6 months post-injection.

Several transport mechanisms have been described for titanium, i.e. systemic dissemination by the vascular system in solution or as particles [42]; lymphatic dissemination as free particles or as phagocytosed particles within macrophages [28, 34]; dissemination of particles to the bone marrow by circulating monocytes or as minute particles by the vascular system [33].

Titanium oxide administered intraperitoneally to rats is deposited in target organs with significant macrophagic activity without undergoing changes in any of its crystallographic properties (anatasa) [35]. This finding shows that TiO_2 did not suffer any chemical alterations, i.e. the titanium–oxygen bond was not broken, and was disseminated as such (anatasa) from the site of injection. Within this context, a feasible transport mechanism would be via macrophages. Thus, the TiO_2 injected

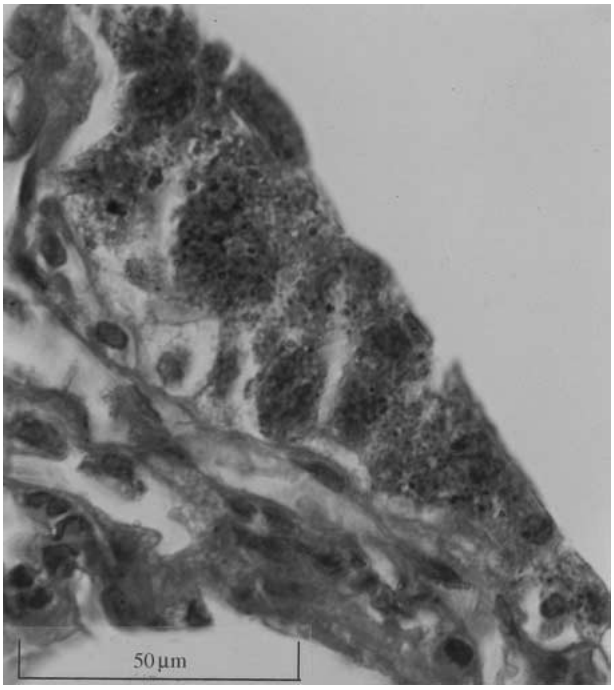

Figure 4 Lung. The cytoplasm of macrophages is loaded with TiO_2 particles (PAS).

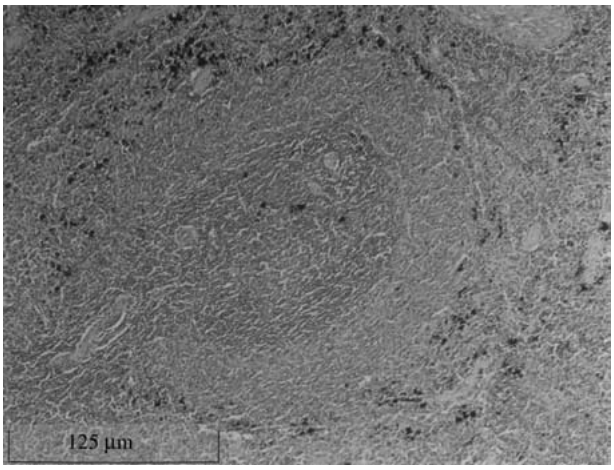

Figure 5 Spleen. Note the presence of TiO_2 material around the lymphoid structures (H–E).

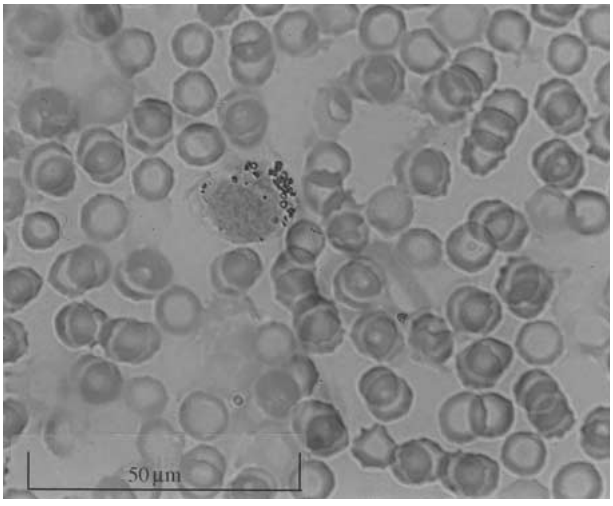

Figure 6 Blood smear showing the cytoplasm of a phagocytic-mononuclear cell loaded with particles (Safranin).

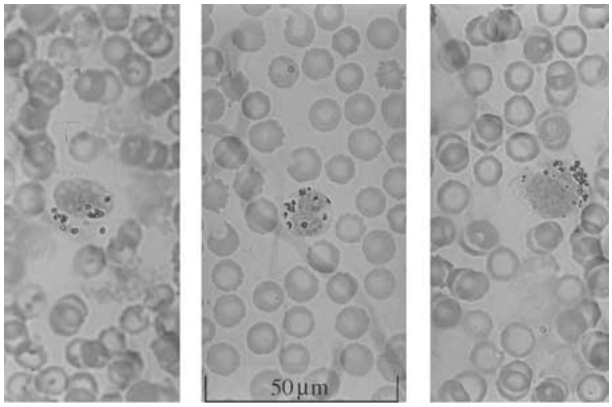

Figure 7 Blood smears. Note the distribution of the deposits in the cytoplasm of the phagocytic-mononuclear cells (Safranin).

intraperitoneally may have been phagocytosed as anatasa by macrophages. Loaded macrophages may have travelled from the site of injection in the lymphatic system to be released, eventually, into the blood stream. This would explain in the present study the presence of phagocytic-mononuclear cells in the blood smears.

Several studies on the bond between metals and proteins have contributed to the knowledge of the dissemination of metals from the site of injection. Nickel, chromium and cobalt would migrate linked to blood cells and/or proteins, in particular albumin, the most abundant protein in serum and tissue fluids [39, 45]. Aluminum would be transported by transferrin [41]. Fifty percent of uranium is transported linked to proteins, 50% to citrates and 50% to carbonates [46]. The fact that metals bond mainly to albumin would explain their widespread presence in the organism. In this way, the metallic ions that result from the process of corrosion would disseminate to tissues, bind to albumin and enter the circulation exerting their effect at remote sites. In the particular case of titanium, little is known about the valence with which it exerts its action, the organic or inorganic nature of its ligands and its potential toxicity [31]. This study confirms the systemic dissemination of titanium by the vascular system via cells. Similarly to other metals, titanium may also be transported by plasma proteins. This issue has not been addressed in the present study. Titanium ions have been detected in organic fluids such as urine and serum by spectrophotometric techniques [28, 31, 47]. The presence of phagocyticmononuclear cells in the blood of patients with prosthesis (coxo-femoral and dental implants, plates and screws to set fractures, metal plates for the reconstruction of large areas) would be indicative of a process of corrosion in the metal structures. The possibility of performing an early diagnosis of this process via the detection of loaded blood cells would be of great value and is an issue of interest for future studies.

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