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Systemic titanium levels in rabbits with a titanium implant in the absence of wear

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All non-noble metals and alloys will release metallic species into the body. This raises the issue of amount and fate, i.e. transport and storage, of these metal dissolution products. For titanium, the nature and extent of these systemic effects remain mostly unknown. In this study we investigated titanium levels in alleged target tissues in rabbits, both with and without a titanium implant functioning in the absence of wear, and compare these results to the limited body of literature concerning systemic levels of titanium. Titanium fibre felts were implanted into the tibia of rabbits. At various time points, lung, spleen, and muscle samples were collected from these rabbits as well as two groups of control rabbits. The samples were analysed for titanium concentration using electrothermal atomic absorption spectrophotometry. The data for the implant groups show that titanium levels in these tissues do not increase in comparison with controls up to 1 y after implantation.

1. Introduction

With current clinical trends, such as longer implantation times and increased surface areas creating a potential for greater metallic release, the incompleteness of the literature in addressing the possible increase in systemic titanium levels associated with the use of titanium-based implants is becoming evident. Ferguson *et al.* [1] were the first to examine systemic titanium levels in rabbit tissues in the presence of a titanium implant. After a 4–6 month implantation period, they determined the amount of titanium in samples of liver, spleen, kidney, lung and muscle. For the majority of rabbits, there was no difference in titanium levels between controls and rabbits with the implants. However, some of the experimental rabbits showed elevated titanium levels in spleen and lung tissue. Inconsistencies prevent the use of these data in making a sound conclusion on the presence or absence of systemic transport of titanium. For example, similarly high levels of titanium were measured in organs of animals that had non-titanium-containing implants.

In 1984, Woodman *et al.* [2] attempted to quantify the amount of titanium released from a Ti–6Al–4V prosthetic segmental replacement in the long bones of baboons. There was no statistical difference between experimental and control animals in serum titanium concentrations. A six-fold increase was measured in the urine of the experimental group (implantation time 36–92 months) compared to controls. There were inconsistencies of titanium levels in local muscle. Lung, spleen, and regional lymph node samples of baboons with implants had consistent increases in titanium levels in comparison to controls. No kinetic data were presented for serum and urine. A kinetic analysis was given for the spleen and lungs. However, interpreting these data is difficult due to several experimental problems. These arose because the original goal of the study was not the determination of metallic release, but the long-term follow up of bony ingrowth. The implant design was complex. It had several parts made of different alloys of titanium. In addition, stainless steel screws were used. Thus, the likely presence of fretting and galvanic corrosion and wear could have been factors that increased the scatter of the data. Additionally, the specimens were non-uniform in size and were implanted in different locations among the animals of the study. It has been shown that corrosion rates of 316L stainless steel implants exhibited a dependence on implantation site [3].

In a more recent study, Lugowski *et al.* [4] determined the amount of titanium in brain, liver, lung, kidney, and spleen tissues in a 2 y rabbit implantation experiment. Because there were no true controls, i.e. rabbits with no implants, the rabbits with non-titanium containing implants were considered as controls. No differences between either the rabbit with a dense Ti–6Al–4V ($n = 1$) disc or the rabbits with a porous Ti–6Al–4V disc ($n = 3$) and the control rabbits were found in any of the analysed tissues. Although the authors have developed careful trace-element procedures [5], this report leaves the understanding of systemic distribution pattern of titanium largely untouched. The use of biased controls and a small

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sample size eliminates any statistical comparison. Furthermore, the implantation procedures, such as the implantation site, are not detailed.

In an effort to limit the form of release to electrochemical phenomena, in this study a titanium porous plug with a high specific surface area was implanted into the tibia of rabbits. At selected times up to 1 y post-operatively, titanium levels in various remotely located tissues were measured and compared to controls. The results of this comparison were used to indicate whether titanium released in the absence of wear is transported to systemic storage tissues to a measurable extent.

2. Materials and methods

A titanium fibrefelt (Tifelt) was chosen as the implant. The highly porous nature of the Tifelt allows a small implant to have a large surface area. The characteristics and fabrication of the Tifelt have been previously described [6, 7]. The disc-shaped implants were 6.35 mm diameter and 2.3 mm thick composed of grade 1 titanium fibres that were 50 μm in diameter. The implants were cleaned and passivated according to ASTM procedure [8] (Standard F86) and sterilized using ethylene oxide gas.

Adult male rabbits, 35 in all and weighing 2.40–3.30 kg, were divided into five groups. NIH guidelines [9] for the care and use of laboratory animals were followed. Seven rabbits received the Tifelt for 1 month, 7 received the implant for 4 months, and 7 received the Tifelt for 12 months. Surface area measurements showed, given the weight range of the rabbits, the implant surface area to rabbit weight ratio was about three times the ratio a "standard" person, who had a cemented hip arthroplasty, would experience. Seven rabbits (shams) underwent the surgical procedure, but did not receive the implant. The final group (controls) were not operated upon. The controls established baseline titanium tissue levels. The shams were used to determine if any titanium contamination occurred as a result of the surgical procedure. Five additional rabbits served as reserves to allow for exclusions due to diseases unrelated to the experiment.

The implantation site was the proximal-medial aspect of the tibia. The surgical procedure has been previously documented [10]. After the appropriate implantation time, or after 12 months for the sham and the control groups, the rabbits were euthanized. During the necropsy, the spleen, gastronemious from the contralateral side with respect to the implant, and left lung were collected. These tissues were sampled because they have been implicated as storage sites of titanium.

Because very low levels of titanium were expected, procedures that prevented contamination of the samples during both collection and processing were used [11–13]. The titanium levels in the tissues were determined using electrothermal atomic absorption spectrophotometry (EAAS). EAAS requires the sample to be in liquid form. Microwave digestion was used to obtain a homogeneous liquid from the solid tissues. Tissues were not lyophilized. While many researchers in the field of trace-element analysis use dry tissue weights, i.e. tissue that has been lyophilized, wet tissues were directly used in this study. The use of wet tissues reduced processing time and avoided possible contamination. Because the weights of wet tissues are unstable due to dehydration, autolysis and other unknown causes, great care must be taken in recording the weight. Dry tissue also allows for homogenization of the sample to account for any possible spatial gradient in [Ti]. Pilot studies to examine this effect did not identify any spatial variation in the titanium content in any tissues. The consistent sampling of specific tissue sections based on anatomical landmarks minimized this possible effect. Recovery studies ensured that no titanium was lost or imparted during the processing. The programme development for EAAS demonstrated that the method of standard additions was necessary for accurate determination of titanium levels.

To test the hypothesis that the two controlled factors, i.e. time and treatment (control, sham, and implant), had no effect on the titanium content, one-way analysis of variance (ANOVA) was performed. If the ANOVA indicated that a difference existed ($P < 0.05$), *post-hoc* comparisons using Student's t test and Tukey–Kramer honestly significant difference (HSD) test were employed to compare individual groups. Because ANOVA is based on a number of assumptions regarding the data, if the tests of these assumptions failed, non-parametric analyses were performed.

3. Results

The means of the measured lung titanium content for each group of rabbits are given in Table I. In calculating the group means, any sample that had a lung titanium content below the 23.9 ng g^{-1} detection limit was set to the detection limit. Two values (one each from the 1 and 4 month implant groups) were determined to be outliers ($P < 0.05$) by the Dixon Q Test. Only eight of the 32 samples tested had lung titanium levels above the detection limit. There were no significant differences with time among the three implant groups. There were also no differences among the control, sham, and any of the implant groups. A χ^2 test verified at the 0.05 level that all groups had values below the detection limit at equal frequencies. There is some uncertainty in performing statistical analysis on groups with a considerable percentage below a detection limit. Regardless, the close grouping of the means and the large number of samples from all of the rabbits below the detection limit indicate that there is no effect of Tifelt implant on lung titanium levels.

TABLE I Lung titanium content

Rabbit group	Ti content (ng g^{-1})	n	Samples below D.L.
Control	25.9 ± 4.54	5	4
Sham	25.0 ± 3.01	7	6
1 Month Implant	24.6 ± 1.68	6	3
4 Month Implant	24.8 ± 1.76	5	3
12 Month Implant	24.3 ± 1.00	7	6

TABLE II Spleen titanium content

Rabbit group	Ti content (ng g^{-1})	n
Control	77.0 ± 24.5	5
Sham	94.3 ± 21.2	4
1 Month Implant	41.8 ± 9.9	5
4 Month Implant	56.7 ± 13.0	6
12 Month Implant	90.1 ± 27.6	6

The means of the measured spleen titanium content for each group of rabbits are presented in Table II. The small size of the rabbit spleen necessitated the use of the entire organ to yield a tissue weight of ~0.5 g required by the digestion procedure. As a result, six samples were not available for analysis due to sample loss during digestion. No spleen titanium contents were below the 28.2 ng g^{-1} detection limit. There were no significant differences among the control, sham, and 12 month implant groups. ANOVA of the implant groups by time indicated a significant difference ($P < 0.05$). Pairwise comparison of the groups with Student's t test ($\alpha \sim 0.05$) showed that the mean spleen titanium content of the 12 month implant group was higher than the mean spleen titanium content of both the 1 and 4 month implant groups. The more conservative Tukey–Kramer HSD pairwise test ($P < 0.05$) confirmed this difference. The control and sham groups also were significantly different from the 1 and 4 month implant groups at the same level.

Because all three of the 12 month groups had titanium spleen contents higher than both the 1 and 4 month implant groups, this increase is unrelated to the Tifelt. If the increased titanium content was the result of passive dissolution of the implant, the elevated titanium levels would have occurred only within the implant group. At first glance, this result may seem puzzling. However, if the physiological function of the spleen is considered, a plausible explanation is found. The spleen is a lymphoreticular organ. As part of the immune system, it is a major site of clearance of micro-organisms and particulate antigens from the bloodstream and of generation of the humoral and cellular responses to foreign antigens [14]. In the general trace-element literature, i.e. in the absence of metallic implants, the spleen has been shown to be an accumulation organ for metals [15]. Although this has not been reported for titanium specifically, this explains the data and gives credence to the belief that the increases are not related to the implant. Further support for this explanation is evident when the titanium levels in the spleen are compared to the titanium levels measured in the other tissues distal to the implant. The spleen had the highest titanium content of all the distal tissues at all time periods.

All 33 distal muscle tissue samples which were analysed had titanium contents below the 25.7 ng g^{-1} detection limit. Even with state-of-the-art instrumentation and carefully developed and verified methods, the titanium content in distal muscle tissues was not detectable. Release of titanium from this implant did not increase the titanium content in distal muscle to values above the detection limit.

4. Discussion

The tissues used to examine systemic distribution of titanium released from a titanium implant did not indicate that systemic accumulation in the absence of wear was occurring to a significant extent. All rabbits had remote muscle titanium contents below the detection limit. The means of the titanium content of lung tissue for all groups were not statistically different ($P > 0.05$). Many of the samples from all groups had lung titanium contents near the detection limit.

The data for the spleen tissue titanium content require a more extensive analysis. The means of the spleen titanium content for the control, sham, and 12 months implant groups were not statistically significantly different. However, the means of these three groups are 1.6 times higher than the mean of the 4 months implant group and 2.2 times higher than the mean of the 1 month implant group. The difference between the 1 and 4 month implant groups was not statistically significant. Because all the 12 month groups (the control and sham groups were followed for 12 months) had elevated mean spleen titanium contents compared to the 1 and 4 month implant groups, this increase does not appear to be related to the implant. As discussed in Section 3, the increase may result from the physiological function of the spleen as part of the lymphoreticular system. This conclusion could be made with greater certainty if 1 and 4 month control and sham data were available. However, in the planning stage of the *in vivo* experiment, a thorough literature search and the pilot study did not indicate any need for 1 and 4 months control and sham data. Regardless of this limitation, the uniform elevation of the mean spleen titanium content for the 12 months groups supports the finding that there is no significant systemic storage of titanium from an implant in the absence of wear.

Irrespective of the type of experimentation, i.e. *in vitro*, *in vivo*, or clinical, it is extremely difficult to compare the results of different trace-metal studies. This is the result of many factors, including: the use of different animal models [16]; the use of different implantation sites [3]; varying implanation times; and the implanation of different surface areas of metals with various surface preparations. Frequently, in the published version of the studies, only minimal details of the methodology are given. For example, key information, such as the surface area of metal, is often not available. Therefore, the following comparison of studies must be viewed with these caveats.

Fig. 1 compares the titanium content for various tissue types in control animals measured in several different studies. Rabbits were used as the animal model in all of these studies, with the exception of Woodman *et al.* [2] who used baboons. In all but one series, control animals were animals without implants of any type. Lugowski *et al.* [4] used rabbits with either hydroxyapatite, Co–Cr–Mo alloy, or alumina implants as controls. In all cases the titanium content was converted into nanograms of titanium per gram of wet tissue. If dry tissue weights were given, the appropriate conversion to wet tissue weights was determined by lyophilization of several samples.

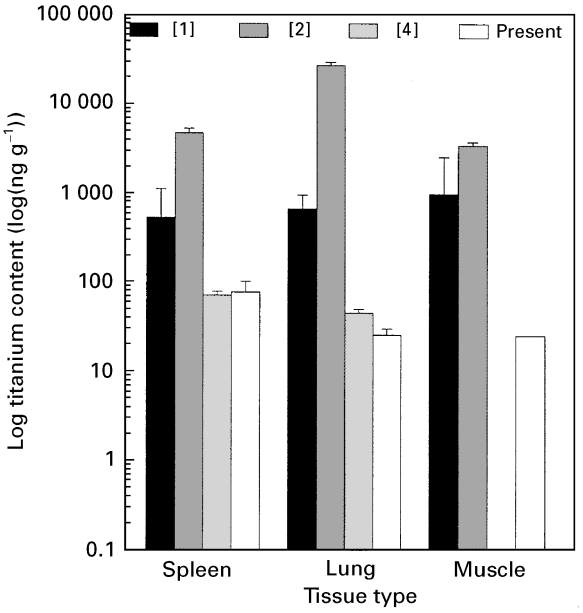

Figure 1 Comparison of control titanium levels in various tissues determined in several studies.

Depending on the tissue, the ratio of wet tissue weight to dry tissue weight ranged from 4 to 5.

In Fig. 1, the titanium content is expressed on a logarithmic scale. The wide range of values necessitated the use of this scale. This figure supports the emphasis of this and other papers that careful contamination control and well-developed methods are essential in trace-element studies. With the exception of the data of Lugowski *et al.* [4], the control data of the other studies are from 1 order to 3 orders of magnitude higher. The agreement between this study and Lugowski *et al.*'s study at the lowest levels ever reported for control tissue titanium contents, ascertains the excellent contamination control achieved in this study.

Table III compares the findings of these same studies in terms of the effect implanted titanium had on the titanium tissue levels. In this table, the effect implanted titanium had on each tissue is demonstrated in terms of the ratio of the experimental group mean titanium level to control group mean titanium level. A value equal to 1 indicates that there was no difference found. It should be noted that although the column for the present study lists brain tissue as not being analysed, pilot studies involving both controls and rabbits with Tifelt implants indicated that the titanium content of brain tissue was not detectable.

In view of the data for the tissues distal to the implant, the results from the present study are similar to the data from the work of Lugowski *et al.* [4]. However, in the present study, sufficient numbers of rabbits were used for valid statistical analysis. Two other studies [1, 2] found systemic accumulation of titanium in the spleen and lung. These differences could be due to a number of factors. The present study used state-of-the-art instrumentation that was not available to the earlier investigators. The methods of the present study were carefully developed and verified. The earlier studies used implants that were not well controlled for wear and fretting corrosion. As a result, transport of particulates through the lymphoreticular system and accumulation in the spleen can have taken place. In this study, a titanium implant in which wear was minimized was used. Any titanium released was primarily the result of passive dissolution processes. The control animals in the other studies appear to have been purchased separately from the implant animals, and consequently, age differences between these groups may have existed. In view of the spleen data of the present study, which indicate that there is an age-dependent effect on titanium content, this could also aid in explaining the discrepancies.

Whereas our implantation model evinces local accumulation [10] and no systemic accumulation, other researchers have reported both local and systemic elevations of titanium. Although local accumulation levels are variable, actually all data for local tissues indicate that there is local accumulation of released titanium. When the release product from titanium implants is a titanium hydroxide, we invoked three related properties of this passive dissolution product of titanium to explain the lack of systemic transport and accumulation [10, 17]: first, the low solubility of the release product, which limits the participation in reactions; second, the limited coordinating capability to cellular chelators as a result of its high acidity; and third, the formation of stable complexes, if and when it interacts, which are not freely transportable.

TABLE III Comparison of the ratios of titanium content for experimental group to control group for several studies

		Ferguson *et al.* [1]	Woodman *et al.* [2]	Lugowski *et al.* [4]	Present study
S.A. to B.W. ratio (cm^2 kg^{-1})		3.6–10.8	45–89	0.60	~10
Number of animals		2–4	5	3	5–7
Implantation time (mon)		1.5–4	12	24	12
Remote tissues	Lung	1.5	5.5	1	1
	Spleen	16	3.3	1	1
	Muscle	n.r.[a]	n.r.	n.r.	1
	Brain	n.r.	n.r.	1	n.r.
Local tissues	Muscle	24	4	n.r.	1.2[b]
	Bone	n.r.	n.r.	n.r.	2[b]
Transport excretion	Serum	n.r.	1	n.r.	1[c]
	Urine	n.r.	6	n.r.	1[c]

[a] n.r. = not reported.
[b] See [10, 13].
[c] See [13, 19].

In addition to this explanation of minimal reactivity such that released titanium stays below a threshold level to trigger a significant response, we can also focus on the data from protein adsorption studies on titanium. These studies have shown that the conformation of fibrinogen was not affected by the titanium surface [18]. If these results are extendable to other proteins and to passive dissolution products, then the binding of proteins to the passive dissolution products in native form could also explain the excellent local tissue response even in the presence of metallic release.

The binding of proteins to passive dissolution products implies that there is a potential for systemic transport and accumulation. However, the data for the *in vivo* study did not find any increases in titanium serum or urine concentrations [19] or titanium content in any of the remote tissues (present study) that could be uniquely associated with the titanium implant. Therefore, if any passive dissolution products-to-protein binding mechanism occurs, there is only minimal transport of the passive dissolution products.

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