BioMetals **12:** 275–280, 1999.

Individual study of chromium in the stainless steel implants degradation: An experimental study in mice

Maria do Carmo Pereira[1,*], Maria de Lourdes Pereira[2] & João Paulo Sousa[3]
[1]*Departamento de Engenharia Química, FEUP, Rua dos Bragas, 4050-123 Porto, Portugal*
[2]*Departamento de Biologia, Universidade de Aveiro, 3810 Aveiro, Portugal*
[3]*INEB-Instituto de Engenharia Biomédica, Faculdade de Engenharia da Universidade do Porto, Praça Coronel Pacheco, 1, 4050 Porto, Portugal*
[*]*Author for correspondence (Fax: +351.2.2000808; E-mail: mcsp@fe.up.pt)*

Received 4 March 1999; accepted 25 June 1999

Key words: biomaterial, chromium, histology, mice organs

Abstract

To study the accumulation and the histological effects in mice organs caused by hexavalent chromium, one of the corrosion products released from AISI 316L stainless steel implants, mice groups were subcutaneously injected with a metallic solution of chromium during a certain period of time. Similar injections were made with HBSS (Hank's Balanced Salt Solution) in other groups to be used as controls. The levels of chromium found in the liver, kidney and spleen of the control and the treated animals were obtained by atomic absorption spectrometry (AAS) and were compared to those obtained by AdSV (adsorptive stripping voltammetry) to test the accuracy of the results. During the experimental period, the liver and spleen showed a progressive and significant accumulation of chromium whereas in the kidney the significant accumulation found after the first week practically remained unchanged during the four weeks. Apparently, the histological analysis of these tissues did not evidence any relevant morphological alteration induced by the chromium accumulations during the four weeks of treatment.

Introduction

Chromium is considered one of the essential metals to the human organism due to a complex of trivalent chromium that has been found to participate in the glucose metabolism (Katz & Salem 1994). However, it is well known that hexavalent chromium causes dermal inflamation and allergy (Young & Houwing 1987) and it is suspected to be a chemical carcinogen (Martin *et al.* 1988; Brien *et al.* 1990; Katz & Salem 1993).

Chromium has been used as an alloying element in joint replacement prostheses for many years (Langard & Hensten-Petter 1981). Several studies showed that chromium and the other alloying metals are released from the implants in high concentrations and are found in blood, urine and also in tissues of patients carrying cobalt-chromium alloys and stainless steel implanted devices (Dobbs & Minski 1980; Black *et al.* 1983; Bartolozzi & Black 1985; Sunderman *et al.* 1989). It has been reported in the literature that the chromium released during corrosion of implants is in the hexavalent form (Merritt *et al.* 1984; Merritt *et al.* 1992; Merritt & Brown 1995) which attaches to albumin (Merritt *et al.* 1984; Merritt & Brown 1985) easily permuting with body fluids and enter in the circulation. There are evidences that hexavalent chromium is reduced to the trivalent form before or during binding to hemoglobin, after which is uptake by cells, i.e., this metal is reduced intracellularly (Bartolozzi & Black 1985).

Most of the metallic materials used in implantology contain significant quantities of at least three elements and each one has distinct biological properties. When stainless steel or other alloys are used, it is extremely difficult to correlate the specific histological features with the characteristics of each specific metal ion. With this in mind, the objective of this work was to investigate the accumulation and histo-

logical effects with time in mice liver, kidney and spleen induced by this metallic specimen after several injections of an hexavalent chromium solution which was produced during the anodic dissolution of the pure metal. These three organs were chosen because of their excretory functions (Ross & Rowrell 1985). Similar studies were done for nickel and iron with the same purpose (Pereira *et al.* 1998a, b) and both metals induced with time several morphological alterations in the mentioned organs. Pure metals were used with the intention to differentiate among the individual effects of chromium, nickel and iron in the stainless steel alloy.

Materials and methods

Chromium dissolution

Discs of chromium Patinal (Merck) were anodically dissolved in Hank's Balanced Salt Solution (HBSS) by imposition of a constant current. The chromium content was determined by flame atomic absorption spectrometry (AAS) and a solution of 140 mg L^{-1} was prepared. The color of the solution was yellow/orange indicating that the valence state of chromium in the physiological solution was hexavalent.

Animals

Male Charles River mice nearly 60 days old weighing 27–34 g supplied from 'Instituto Gulbenkian de Ciências', Portugal, were selected as the laboratory animal model to administer the chromium solution. They were housed in groups of four per cage and food and water were given *ad libitum*. During one week the animals were allowed to equilibrate relatively to their new environment and diet.

Chromium injections and mice sacrifice

0.50 mL of the chromium solution was subcutaneously injected in the back dorsal surface at days: 0–group I; 0 and 7–group II; 0, 7 and 14–group III and 0, 7, 14 and 21–group IV. Each group was composed of eight animals. A similar design was used for the control mice which only received HBSS. The body weight of the animals was registered at the day of the first injection with chromium and at the day of sacrifice. The animals were sacrificed after a week of the last injection, under ether anesthesia. By surgical incision in the abdominal cavity the liver, kidney and spleen were removed. Small fragments of the organs were used for histological studies and the remaining part was stored in PTFE bottles and kept in a freeze chamber at -20 °C for posterior quantitative analysis. During the dissection and in posterior treatment, the organs were not touched with any metallic instrument to insure no chromium contamination. All glassware and polyethylene bottles used were soaked in solutions of HNO_3 20% for several hours and then rinsed with an excess of water and left to dry.

Mineralisation procedure

Approximately 1.0 g of each organ studied was placed into previously weighed vessels and then dried in a microwave oven model MDS 2000 CEM. Inside the vessels 1.50 mL of HNO_3 Suprapur from Merck was added per 100 mg of dry weight (Pereira *et al.* 1997). The microwave digestion proceeded during 30 min. After that, the resulting solutions were evaporated with the addition of 1.0 mL of $HClO_4$ (Merck) per 0.50 g dry mass until perchloric acid fumes were no longer observed. The residue was then diluted with ultra pure water to an appropriate volume.

Analytical procedure

Solutions prepared in this way were analyzed by graphite furnace atomic absorption spectrometry. The spectrometer used was from Perkin Elmer Model 4100ZL in conjunction with pyrolytic THGA (transverse heated graphite atomizer) tubes and autosampler. $Mg(NO_3)_2$ was used as the matrix modifier. The standard addition method was used to quantify the chromium concentration in this type of matrix. All the measurements were performed in triplicate with four standard additions.

Histological analysis

Small fragments of each organ were fixed in Bouin's solution, embedded in paraffin and sections with approximately 3–5 μm were made in a microtome. After staining with Hematoxylin-eosin, sections were then dehydrated in an ethanol series and mounted in Canada Balsam. Observations and photographs were made using a Nikon optical microscope.

Statistical analysis

Statistical analysis of the significance of the differences between all groups injected by chromium and

the control ones were carried out by the Student's *t* method. The acceptable level of significance was at $P<0.01$.

Results

Morphological observations

The mean values of body weight for each group expressed in grams plus the standard deviations were respectively: Group I–23.99 ± 3.60 and 24.94±3.72; Group II–30.98 ± 2.96 and 33.11 ± 2.57; Group III–33.03±2.60 and 30.90±1.73; Group IV–33.86±4.22 and 33.45 ± 0.37. The differences between the body weights at the day of sacrifice and the initial weight were not statistically significant ($p > 0.05$) and they are very similar with the control animals.

No mortality was observed during the animal treatment neither signs of toxicity associated with chromium administration. The macroscopic appearance of the removed organs in all groups seemed to be apparently similar to the control ones.

Chromium analysis

The concentration of chromium found in the studied organs is presented in Table I. The values were expressed in μg g^{-1} dry tissue. To check the accuracy of these results some solutions were analyzed by AdSV (Pereira *et al.* 1997). The differences found by both methods were not statistically significant, the values differed only by 1.2 to 6.1% indicating a good accuracy of the results. Comparing the means of the chromium levels in the control organs *versus* the treated animals, there was a significant elevation ($p < 0.01$) of chromium concentration in the three organs studied during the experimental assay. The levels of chromium in the control mice organs remained essentially unchanged.

Chromium accumulation in the liver, kidney and spleen

Figure 1a shows a progressive and significant increase ($p < 0.01$) of chromium concentration with time when compared to the normal values found in control animals. At day 7 the chromium levels were already approximately twenty times higher than the control group. At day 28 the chromium accumulation was approximately forty times higher compared to the control group, i.e., twofold compared with day 7.

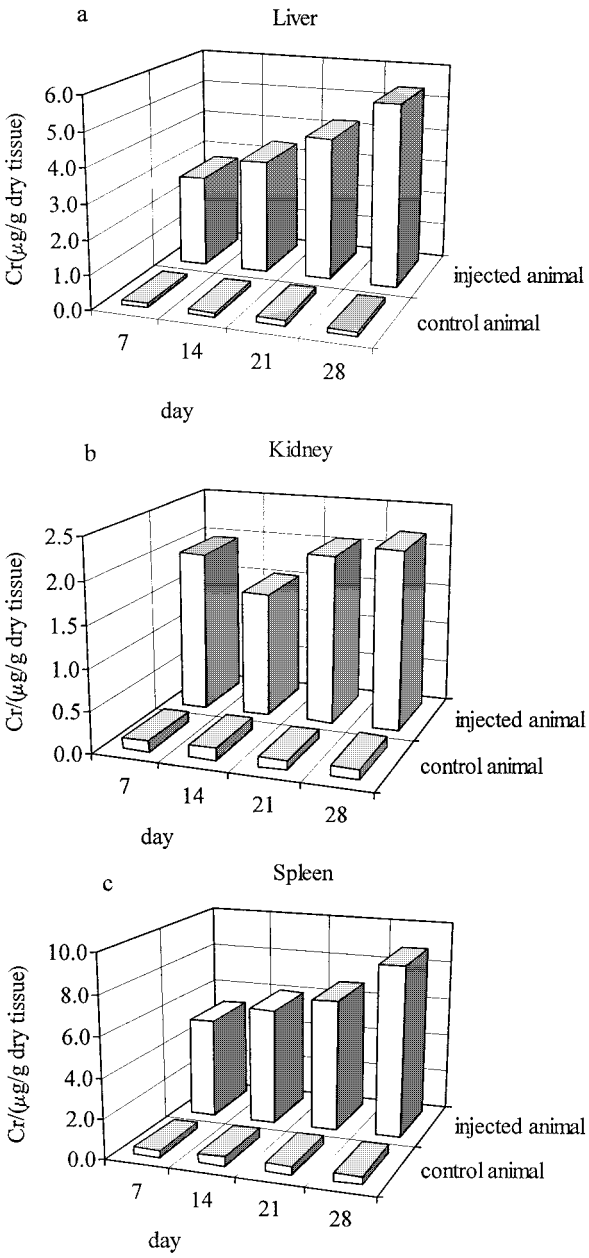

Figure 1. Concentration of chromium found in mice at the days of sacrifice after injection of a chromium solution: (a) liver; (b) kidney; (c) spleen. The animals were sacrificed at days 7, 14, 21 and 28.

It is possible to see a significant increase ($p < 0.01$) of chromium concentration in the kidney of animals injected with this metal and the control ones with time (Figure 1b). The rise was about fifteen times the normal values found in this organ and the chromium quantity accumulated by the kidneys during

Table 1. Results obtained in mice organs by AAS following injections of chromium
The values are expressed in μg g^{-1} of dry weight

	Liver	Kidney	Spleen
7 days			
Control	0.119 ± 0.003	0.127 ± 0.004	0.357 ± 0.007
Injection	2.67 ± 0.10	1.95 ± 0.08	5.07 ± 0.15
14 days			
Control	0.106 ± 0.003	0.153 ± 0.006	0.444 ± 0.009
Injection	3.33 ± 0.10	1.52 ± 0.05	5.94 ± 0.12
21 days			
Control	0.159 ± 0.005	0.107 ± 0.004	0.423 ± 0.006
Injection	4.20 ± 0.08	2.05 ± 0.04	6.70 ± 0.19
28 days			
Control	0.125 ± 0.004	0.124 ± 0.003	0.401 ± 0.005
Injection	5.37 ± 0.13	2.08 ± 0.04	8.75 ± 0.25

Mean for at least three separate determinations and their standard deviation.
Each control and injected group was composed of 8 animals.
The differences in results with both methods are not significant at $p > 0.01$.

the first week practically remained unchanged during the posterior treatment time.

Figure 1c shows a progressive augment of chromium concentration in the spleen of animals injected with this metal compared with the control animals, demonstrating a significant accumulation of chromium in this organ ($p < 0.01$). At day 7 the chromium concentration was fourteen times more elevated than the control organs. At day 28 the accumulation increased approximately twenty times compared to the control organs.

The histological sections of liver, spleen and kidney from all groups that were injected with chromium were similar to the control groups. In most of tissues analyzed the morphological aspects do not present degenerative features.

Discussion

The quantity of chromium initially administered in mice was based in previous published results of analyses of human tissues adjacent to stainless steel implants, the chromium concentration ranged between 210 and 2700 mgL^{-1} (Pohler 1983). In this study each injection contained 140 mgL^{-1} of chromium and corresponded to a simulated corrosion rate of 330 μg kg $^{-1}$ day^{-1}.

After one week from the first injection, the quantity of chromium accumulated in the liver, spleen and kidney organs was approximately 1.4 μg per animal and the quantity initially injected was 70 μg. The Group IV suffered four injections corresponding to 280 μg of chromium and retained approximately 2.5 μg. Thus, only nearly 1% of the total injected chromium was accumulated in these organs. From the literature, it seems that one part of this metal tends to remain at the impregnation site once chromium is cell binding (Brown *et al.* 1993) and from the same study 67.3% of the injected chromium was recovered in urine and 8.7% in organs. However, the major part of the injected chromium appeared to be excreted mainly by the kidneys: approximately 80% of an injected dose have been recovered in urine (Burrows 1983).

As was referred, only ca. 1% of the chromium initially injected remained in the organs and provoked a significant rise in the levels when compared to the organs of the control animals. Thus, chromium quantity accumulated by the kidneys during the first week practically remained unchanged during the posterior treatment time. This suggests an impregnation of the kidney cells with chromium producing a slow, long-term release and apparently this organ was incapable of a rapid elimination by urine. From a study of Bartolozzi *et al.* (1985) in patients that received conventional polymethilmethacrylate cement cobalt-chromium alloy, a post-operative increase excretion of chromium by urine was observed in conjunction with serum rise suggesting that there is a saturation of the urinary excretory pathway. A progressive accumulation of this metal was observed in the spleen and liver indicating a certain storage capacity of these

organs and this ability was confirmed by the inexistence of histological alterations in these organs. Smith and Black in 1985 implanted 316L stainless steel in male New Zealand white rabbits and found that at the end of the seven months of implantation the chromium concentration in the liver was 85% more elevated compared to the control animals.

The higher accumulation of chromium observed in the studied organs appeared to be associated with a greater binding of chromium by the red blood cells as suggested by several studies (Merritt & Brown 1985; Kargacin *et al.* 1993). It is accepted that corrosion implants lead to release of biologically active hexavalent chromium (Yang *et al.* 1994; Kargacin *et al.* 1993). These results are in agreement with other study in Syrian hamsters (Merritt *et al.* 1989) that received four doses of 117 μg of chromium, 90 μg nickel and 94 μg cobalt at monthly intervals. Chromium levels were elevated in all organs (liver, lung, spleen, kidney) compared to control. Chromium was found in the red blood cell and it was eliminated in the urine very slowly (Brown *et al.* 1988).

Apparently, it is possible to deduce that chromium is not the metal of concern for short term stainless steel implants as it did not induce morphological alterations in the mice organs mentioned. However, further studies must be carried out for longer periods of treatment, to observe whether or not it causes histological alterations.

References

Bartolozzi A, Black J. 1985 Chromium concentrations in serum, blood clot and urine from patients following total hip arthroplasty. *Biomaterials* **6**, 2–8.

Black J, Maitin EC, Gelman H, Morris DM. 1983 Serum concentrations of chromium, cobalt and nickel after total hip replacement: a six month study. *Biomaterials* **4**, 160–164.

Brien WW, Salvati EA, Healey JH, Bansal M, Ghelman B, Betts F. 1990 Osteogenic sarcoma arising in the area of a total hip replacement. *J Bone Joint Surg* **72-A**, 1097–1099.

Brown SA, Farnsworth LJ, Merritt K, Crowe TD. 1988 In vitro and in vivo metal ion release. *J Biomed Mater Res* **22**, 321–338.

Brown SA, Zhang K, Merritt K, Payer JH. 1993 In vivo transport and excretion of corrosion products from accelerated anodic corrosion of porous coated F75 alloy. *J Biomed Mater Res* **27**, 1007–1017.

Burrows D. 1983 *Chromium: Metabolism and Toxicity*, CRC Press, Boca Raton, Florida.

Dobbs HS, Minski MJ. 1980 Metal ion release after total hip replacement. *Biomaterials* **1**, 193–198.

Kargacin B, Squibb KS, Cosentino S, Zhitkovich A, Costa M. 1993 Comparison of the uptake and distribution of chromate in rats and mice. *Biol Trace Element Res* **36**, 307–318.

Katz SA, Salem H. 1993 The toxicology of chromium with respect to its chemical speciation: a review. *J Appl Toxicol* **13**, 217–224.

Katz SA, Salem H. 1994 *The Biological and Environmental Chemistry of Chromium*, VCH Publishers.

Langard S, Hensten-Pettersen A. 1981 Chromium toxicology. In: Williams DF. eds. *Systemic Aspects of Biocompatibility*. Boca Raton, Florida, CRC Press, 143.

Martin A, Bauer TW, Manley MT, Marks KE. 1988 Osteosarcoma at the site of total hip replacement. *J Bone Joint Surg* **70-A**, 1561–1566.

Merritt K, Brown SA. 1985 Biological effects of corrosion products from metals. In: Fraker AC. Griffin CD. eds. *Corrosion and Degradation of Implant Materials: Second Symposium*. Philadelphia, American Society for Testing and Materials, 195–207.

Merritt K, Brown SA. 1995 Release of hexavalent chromium from corrosion of stainless steel and cobalt-chromium alloys. *J Biomed Mater Res* **29**, 627–633.

Merritt K, Brown SA, Sharkey NA. 1984 Blood distribution of nickel, cobalt, and chromium following intramuscular injection into hamsters. *J Biomed Mater Res* **18**, 991–1004.

Merritt K, Brown SA, Sharkey NA. 1984 The binding of metal salts and corrosion products to cells and proteins in vitro. *J Biomed Mater Res* **18**, 1005–1015.

Merritt K, Crowe TD, Brown SA. 1989 Elimination of nickel, cobalt, and chromium following repeated injections of high dose metal salts. *J Biomed Mater Res* **23**, 845–862.

Merritt K, Fedele CD, Brown SA. 1992 Chromium 6+ or 3+ release during corrosion; and in vivo distribution. In: Doherty PJ. *et al.*, eds. *Biomaterial- Tissue Interfaces*, *Advances in Biomaterials* **10**, 49–53.

Pereira MC, Faria JL, Reis ML, Sousa JP. 1997a Nickel quantification in mice organs by adsorptive cathodic stripping voltammetry using microelectrodes. *Electroanalysis* **9**, 150–154.

Pereira MC, Pereira ML, Sousa JP. 1997b Adsorptive stripping voltammetric measurements of chromium accumulation in mice organs using mercury film microelectrodes. *Electroanalysis* **9**, 941–944.

Pereira MC, Pereira ML, Sousa JP. 1998a Evaluation of nickel toxicity on liver, spleen and kidney of mice after administration of high-dose metal ion. *J Biomed Mater Res* **40**, 40–47.

Pereira MC, Pereira ML, Sousa JP. 1998b Adsorptive stripping measurements of iron accumulation in mice kidney using microlectrodes and histological features. *J Trace Elements Med Biol* **12**, 50–55.

Pohler OEM. 1983 Degradation of metallic orthopedic implants. In: Rubin LR, ed. *Biomaterials in Reconstructive Surgery*, The C. V. Mosby Company; 158–228.

Ross MH, Rowrell LJ. 1985 *Histology a Text and Atlas*, Williams & Wilkins.

Smith GK, Black J. 1985 Estimation of in vivo type 316L stainless steel corrosion rate from blood transport and organ accumulation data. In: Fraker AC. Griffin CD, eds. *Corrosion and Degradation of Implant Materials; Second Symposium*, American Philadelphia, Society for Testing and Materials; 223–247.

Sunderman Jr FW, Hopfer SM, Swift T *et al.* 1989 Cobalt, chromium, and nickel concentrations in body fluids of patients with porous-coated knee or hip prostheses. *J Orthop Res* **7**, 307–315.

Yang J, Black J. 1994 Competitive binding of chromium, cobalt and nickel to serum proteins. *Biomaterials* **15**, 262–268.

Young E, Houwing RH. 1987 Patch test results with standard allergens over a decade. *Contact Dermatitis* **17**, 104–107.