Release and uptake of cobalt from cobalt–chromium alloy implants

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Cobalt–chromium alloy implants were placed subcutaneously in the neck region of mice, and the animals were killed after 8 weeks. The cobalt concentration was high in the connective tissue of the capsule formed around 5 out of 10 cobalt–chromium implants. In nine other tissues analyzed no statistically significant increases in the cobalt concentration were detected. The surgical procedure and the carrying of an implant did not influence the weight increase of the animals during the period of the experiment. □ *Dental materials; flameless atomic absorption; mice*

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Cobalt is a heavy metal whose toxic properties have been discussed by several authors (1, 2, 10, 17, 21, 23, 24, 29, 39, 43, 44, 46). In other papers the allergic risks involved in using cobalt have been discussed (15, 16, 18, 19, 28, 33–36, 42). In Sweden cobalt is listed as a sensitizing heavy metal (47). When cobalt was tested epicutaneously, there were positive reactions in 8.5% of tests on 843 patients referred to the Department of Dermatology at the Regional Hospital in Umeå during 1978 for dermatological examinations of contact allergy (20).

In medicine and odontology cobalt–chromium alloys are used as substitutes for lost or defective tissues. These alloys, which contain up to about 60% cobalt (37), are mainly used in odontology for removable partial dentures and in certain cases for implants.

Cobalt–chromium alloys used for implants corrode in vivo (9, 14, 45), and studies in rabbits have shown an increase of the cobalt content in muscle tissues around implants (14). Shell splinters containing cobalt in the shoulder–arm region of a patient have caused cobalt allergy (36). An increased cobalt content in the blood of patients with metal hip joint restorations has been reported (11).

Information about the release of cobalt from implants and its further distribution in the mammalian body is scanty. The purpose of the present study was to investigate whether the cobalt possibly released from a subcutaneous cobalt–chromium implant in mice is taken up by certain tissues.

Materials and methods

Animals and implants

The experimental animals used were 30 non-pregnant female mice (N.M.R.I. strain) approximately 8 weeks old and weighing about 20 g (Anticimex, Stockholm, Sweden). Three animals were taken from 10 litters. They were housed in acrylic cages with stainless steel covers and had free access to ordinary tap water and a standard conventional pellet diet (210 Anticimex). The experimental animals were divided into three groups of ten so that one animal from each litter was placed in each group: group I, control mice; group II, experimental mice given a silicon rubber implant (10×6×2 mm); and group III, experimental mice given a cobalt–chromium alloy implant (10×6×2 mm).

The cobalt–chromium implants were cast in Vitallium® (Austenal A.G., Cologne, FRG) with a cobalt content of 62.5 weight

percent (8) and the silicon rubber implants were made in Dow Corning® 382, Medical Grade Elastomer (Dow Corning, Brussels, Belgium) with a cobalt content of about 2 μg/g. Both materials were handled in accordance with the directions of the manufacturers. One side of the cobalt–chromium casting was finished electrolytically in a solution from Howmedica (Glänzbad-Flüssigkeit, Austenal A.G., Cologne, FRG), and the other side was polished with carborundum–rubber discs and polishing compound (item 653, Howmedica) on small brushes (Robinson type). The electrolytic solution and the polishing compound, according to the manufacturer, contain no cobalt. Afterwards the castings were washed ultrasonically in a mixture of ammonia and soap solution (Anti Septon, Grummebolagen, Ekenäs, Stockholm, Sweden). No cobalt could be detected in this mixture by means of atomic absorption spectrophotometry. The castings in the present study were finished in the same manner as castings used in removable partial dentures, thus producing the same surface structure properties. The implants were placed subcutaneously in the neck region via a sagittal incision 8–10 mm long, and the operation was performed under pentobarbital sodium solution (Mebumal®; ACO, Gothenburg, Sweden) anaesthesia. The incision was closed with stainless steel agraffes 7.5×1.75 mm (von Wachenfeldt 37630-7 Stille AB, Stockholm, Sweden), which were removed after about 1 week when primary healing was established. The control mice in group I were not subjected to any surgical procedure. Eight weeks after the insertion of the implants the animals were killed. Samples were taken from the heart, lung, thymus, parotis, liver, spleen, pancreas, kidney, and teeth. The sample procedure used has been described earlier (5). Various errors were analyzed and found to be small (5). Samples were also taken from the connective tissue capsules, which had formed around the implants. The samples were dried for 24 h at 110°C and were ashed for 2 days at 550°C. Both the dry weight and ash weight of the samples were determined. 0.3 ml of 3 mol/l HNO_3 (ultrapure) was added to the test tubes with the dry-ashed tissues. After being covered with Parafilm 'M', the tubes were allowed to stand for 7 days and were shaken daily.

Atomic absorption spectrophotometry

The cobalt analyses were made by flameless atomic absorption spectrophotometry (AAS) at a wavelength of 240.7 nm. A Varian AA-6 spectrometer supplied with a carbon rod atomizer model 63 (CRA 63) was used. Facilities for close temperature control of the graphite tube had been installed as described by Lundgren (32). A 5-μl volume of each tissue sample solution was injected into the graphite tube with a micropipette (Unimetric, 0–10 μl, Unimetrics Corp., Anaheim, Calif., USA), and drying (40 sec at 100°C), ashing (45 sec at 750°C), and atomization (2.5 sec at 2500°C) were performed. All samples were analyzed twice. Calibration curves were drawn from peak height signals obtained for standard solutions of $CoCl_2$. The sensitivity of cobalt with the method is 0.8 ng/ml (0.8 ppb), and detection limit, defined as twice the noise level, is 2 ng/ml. The cobalt content in all tissues was expressed in parts/10^9 (ng/g) dry weight.

The flameless atomic absorption method used has been described by Lundgren (32) and the peak reader module used for registration of the absorbance signals by Lundberg (30). To achieve the largest possible signals, the ashed tissue samples were dissolved in a minimum amount of HNO_3, and the largest recommended volume (5 μl) was used in the analysis. The influence of the different matrixes on the cobalt signal was investigated. For teeth, a decrease in signal of 50% was observed, whereas for the other organs the decrease was less than 11%. The results reported in Table 1 have not been corrected for these matrix interferences, because a specific tissue shows the same decrease in signal for all animals, and this study is comparative (31).

Using flameless atomic absorption we found no detectable amount of cobalt in the ultrapure HNO_3 used in the present study. To investigate the possibility of a potential cobalt content in the glassware, Pyrex test

Table 1. The concentrations of cobalt in tissues of control mice and of mice with implants of silicone or cobalt–chromium alloy. Values in parts/10^9 dry weight, ng/g. n = number of mice, $\bar{x}$ = mean value, S = standard deviation of the mean, CV = coefficient of variance, N.D. = not detectable

	Controls (group I), n = 10			Silicone implants (group II), n = 10			Cobalt chromium alloy implants (group III), n = 10		
	$\bar{x}$	S	CV	$\bar{x}$	S	CV	$\bar{x}$	S	CV
Heart	306.9	62.7	20.44	343.4	117.4	34.19	318.4	33.1	10.42
Lung	106.1	28.5	26.91	126.4	39.3	31.10	121.4	33.8	27.90
Thymus	127.9	74.0	57.84	112.6	40.9	36.29	181.1	111.4	61.52
Parotis	96.8	57.6	59.51	127.0	70.4	55.45	91.1	45.4	49.81
Liver	256.9	50.5	19.66	263.0	54.0	20.54	294.3	46.8	15.91
Pancreas	145.5	74.7	51.35	140.4	36.8	26.18	133.3	30.1	22.58
Kidney	1,465.5	147.7	10.08	1,493.6	171.5	11.48	1,524.8	270.3	17.73
Spleen	N.D.			N.D.			N.D.		
Teeth	N.D.			N.D.			N.D.		

tubes, washed, unwashed, or burned at 570°C, respectively, were filled with 1 ml 1 mol/l HNO_3 and allowed to stand for 6 days. No detectable amount of cobalt was found in the test tube content. To discover any possible cobalt content in Parafilm 'M', three pieces, 1 cm^2 in total, were put into a test tube with 1 ml 1 mol/l HNO_3. After 14 days no detectable amount of cobalt was found in the HNO_3 solution by flameless atomic absorption.

Any possible loss of cobalt from the samples during the drying-ashing procedure was studied by using ^{58}Co as a tracer injected in three mice, which were killed 1 day after injection. The samples were taken in the usual manner, and the ^{58}Co activities were measured both before and after the drying-ashing procedure described above. After the ashing procedure the samples were dissolved by adding 0.3 ml 3 mol/l HNO_3 to the test tubes and covering them with Parafilm 'M'. They were allowed to stand for 7 days, being shaken daily. The content of the test tubes was filtered (Munktell 1F). Then the tubes were washed with 10 ml pure de-ionized water (Millipore), which was also filtered. The filter papers were washed with 20 ml more of pure de-ionized water and put into other tubes. Scintillation measurements were performed on the empty test tubes, to determine the loss of cobalt to the glass, as well as on the tubes with the filters, to determine the amount of cobalt in undissolved ash. The mean value loss of cobalt to the test tubes ranged from 0.8% to 3.4% for all tissues, and the corresponding figures for ash were 3.1% to 5.6%, except for the pancreas, which showed a mean loss of 21.0%. This high value for the pancreas may be due to the formation of cobalt compounds, which are hard to dissolve, to an inclusion of cobalt into particles, making cobalt non-attackable by the acid used, or to the possibility that cobalt can undergo a reduction and become metallic. That the type of tissue is an important determinant in the loss of trace metals through retention in an acid-insoluble form in the ashing vessel is in agreement with the findings of Koirtyohann & Hopkins (25). The high loss of cobalt when ashing pancreas does not influence the results, because in the present study statistical comparisons were only made between the same tissues from various groups. The mean value loss of cobalt to the air was estimated to 1%. The loss of cobalt during the ashing procedure has been studied by various authors with contradictory results (for review, see Ref. 38). None of these studies used the same determinants as the present study, making direct comparison complicated.

Statistical method

Interindividual differences between the mean values for the cobalt content of the tissues for the various groups were tested

statistically for two independent samples in accordance with Wilcoxon's test. This test was also used in the statistical analysis when the groups were compared for increases in weight that occurred during the 8 weeks of the study. P values less than 0.01 were taken to show a statistically significant difference.

Results

Any cobalt content in the spleen and teeth could not be quantified. The results in Table 1 show that in all groups the kidney had the highest cobalt concentrations. With regard to the other tissues the lung, thymus, parotis, and pancreas showed somewhat lower cobalt concentrations than the heart and liver. No statistically significant differences between the groups with regard to the cobalt content of the above-mentioned tissues were obtained. When the animals were compared with reference to the cobalt content of the connective tissue capsule formed around the cobalt–chromium alloy implant (Table 2), there were extremely wide variations, with values ranging from those far in excess of the figure obtained for the kidney to undetectable. Only two animals showed measurable but comparatively low cobalt concentrations in the connective tissue capsule around the silicon implants (Table 2). A statistical comparison was made within the group that had received cobalt–chromium alloy implants, comparing those animals that had connective tissue capsules around the implants with high (five mice) and with no detectable (five mice) cobalt concentrations, respectively, with regard to the cobalt content in the heart, lung, thymus, parotis, liver, pancreas, and kidney. No statistically significant differences were obtained.

Table 2. The concentration of cobalt in the connective tissue capsules around the implants of silicone or cobalt–chromium alloy in mice. Values in parts/10^9 dry weight. ND = not detectable

Silicone implants	Cobalt–chromium alloy implants
128	2,520
63	900
	766
	13,350
	795
8ND	5ND

The initial weight of the mice was about 20 g each. Concerning the increase in weight during the experimental period the following mean values ($\bar{x}$) and standard deviations (S) were obtained: cobalt–chromium alloy implant group, $\bar{x} = 3.38$ g, $S = 1.11$ g; silicone implant group, $\bar{x} = 2.85$ g, $S = 1.28$ g; control group, $\bar{x} = 4.34$ g, $S = 1.77$ g. A statistical comparison between the groups did not show any statistically significant differences in weight increase.

Discussion

Checking the weight increase of a laboratory animal is one of the most fundamental ways of marking the 'well-being' of the animal (12). There were no statistically significant differences when the increase in weight of the control group was compared with that of the other groups. Thus neither the act of implantation nor the implants themselves seemed to influence the well-being of the experimental animals in the present study.

In this study the thickness of the capsule around the implant showed variations. The aim was neither to study the formation rate of the capsules around the implants nor to compare the two implant groups with regard to potential differences in the quality of the capsule. However, it should be noted that in a study of rats with cobalt–chromium implants placed in subcutaneous muscle tissue a strong cell reaction with necrotic regions could be seen around the implants 16 days after the insertion (9). After 25 days a fibrous capsule was seen to be forming around the implants, and after about 75 days the capsule was almost complete but not uniform. In another study (27) the thickness of the connective tissue capsule around a metal implant in general was shown to be proportional to the amount of ionized metal and thus a sign of tissue reaction.

In the present study there was a great

difference between the kidney and the other tissues—capsules excluded—with regard to cobalt contents. Cobalt concentration values of various mammalian tissues have previously been reported (13, 40, 41). However, data are lacking for laboratory mice. Thus no comparison is possible with other studies in this respect.

In previous studies cobalt–chromium implants have been shown to corrode in vivo (9, 13, 14, 45). In one of these studies (13) the cobalt content increased in several tissues of rabbits with plugs of cobalt–chromium alloy embedded into the skeletal muscles. The increase was higher after 6 weeks than after 16 weeks. In the present study the high cobalt concentrations in the connective tissue capsules formed around certain cobalt–chromium implants show that cobalt may be released owing to electrochemical corrosion. The present study could not determine whether the cobalt in the capsules of two silicone implant animals emanates from the silicone material itself or from, e.g., food or water.

The registered concentrations of cobalt were those present at the time of death of the mice and consequently do not reflect the total amount of cobalt released from the cobalt–chromium alloy implants. This discussion has been further developed by Bergman et al. (7). In certain connective tissue capsules formed around the implants of cobalt–chromium alloy no cobalt could be quantified. This may indicate differences in the corrosion stability of the implants. Corrosion stability of base metal alloys depends on the presence of a protective film that markedly lowers the rate of corrosion (22). Various reasons for the disruption of the passivating film have been discussed (3, 26). The variation in capsule content of cobalt may also be a measure of different turnover rates for cobalt among the animals owing to biological variations.

This study has shown that cobalt can be released from subcutaneous implants of cobalt–chromium alloys and accumulated in the capsules surrounding the implants. Since the oral milieu can be more or less corrosive (e.g. Refs. 4, 6), it would be worthwhile also to study the potential release of cobalt from cobalt–chromium alloy constructions in the oral cavity.

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