

Patch test reactivity to a cobalt–chromium–molybdenum alloy and stainless steel in metal-allergic patients in correlation to the metal ion release

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Nickel, chromium, and cobalt released from stainless steel and CoCrMo alloys have been postulated to trigger hypersensitivity reactions. The objective of this study was to assess the ion release from a CoCrMo alloy and stainless steel *in vitro* and the cutaneous reactivity to it by patch test. 52 metal-allergic patients and 48 non-allergic controls were patch tested to stainless steel and CoCrMo discs. In addition, using atomic absorption spectrometry, the release of nickel, cobalt, and chromium from both materials was assessed upon 2-day exposure to distilled water, artificial sweat (AS), and cell culture medium. There was low nickel ion release from stainless steel (0.3–0.46 µg/cm²/2 days) and CoCrMo discs (up to 0.33 µg/cm²/2 days) into the different elution media. Chromium release from the 2 materials was also very low (0.06–0.38 µg/cm²/2 days from stainless steel and 0.52–1.36 µg/cm²/2 days from CoCrMo alloy). In contrast, AS led to abundant cobalt release (maximally 18.94 µg/cm²/2 days) from the CoCrMo discs, with concomitant eczematous reaction upon patch testing: 0 of the 52 metal-allergic patients reacted to stainless steel discs and 5 of the 52 patients to CoCrMo discs (all 5 patients were cobalt allergic and 3 also nickel and chromium allergic). None of the controls reacted to the discs. Apart from nickel being a focus of allergological research, our results point to the possibly underestimated association of cobalt release and potential hyperreactivity to CoCrMo alloy.

Key words: allergy; arthroplasty; cobalt; implant; metal release; nickel. © Blackwell Munksgaard, 2007.

Accepted for publication 13 February 2007

Cr, Co, and particularly Ni are among the most frequent elicitors of delayed-type hypersensitivity (DTH) in the general population. The overall sensitization rates may range between 13% (Ni) and 1.1% (Cr), with further differences based on age and sex (1). Eczema to metallic daily use articles – particularly if in prolonged or repeated skin contact like jewellery, earrings, and watch bands – is well known in patients allergic to Ni, Cr, or Co. With regard to implantation of CoCrMo alloys and stainless steel as widely used materials, biocompatibility aspects mostly focus on wound/fracture healing and long-term performance of arthroplasty (2–4). CoCrMo-based alloys release Co, Cr, and Ni into the surrounding tissues, leading to the presence of these metal ions also in the blood and urine of the respective patients. This

has particularly been observed in association to wear and corrosion products generated by joint replacement prostheses (5, 6). Resulting biological effects were mostly described and examined as osteolysis linked to foreign body-like reactions (7). Specific DTH to implanted metals has been described under the picture of local or generalized eczema, urticaria, and impaired wound or fracture healing (8–11). Peri-implant tissue retrieved from aseptically loosened metal-to-metal arthroplasties has in part been reported to show characteristics of DTH reactions like lymphocytic infiltrates and pseudo-lymph follicles (12–14). The aim of the present study was to evaluate the potential of cutaneous reactivity to stainless steel and CoCrMo discs in patients with a proven contact allergy to Ni, Co, or Cr. Furthermore, this reactivity was

compared with the amount of released Ni, Co, or Cr in eluates prepared from such metal discs.

Materials and Methods

Metal discs

The stainless steel and CoCrMo alloy – used as metal discs in this investigation – are commonly applied in permanent implants like hip stems. Both materials were commercially obtained from Aesculap, Tuttlingen, Germany. The stainless steel used here (according to ISO 5832–9) was a more corrosion-resistant type because of its nitrogen-alloyed manufacturing. The CoCrMo alloy (according to ISO 5832–12) was used as wrought material, having a more homogeneous structure but identical chemical gross composition as the cast type. The chemical composition of the alloy and the stainless steel used here is shown in Table 1 and is consistent with the requirements of the respective ISO standards (also given in Table 1). The composition of the test samples was assessed by Aesculap using emission spectrometry. The discs of the above-described standard alloys were further manufactured by Aesculap. This manufacturing included machining (turning and slip grinding) to a diameter of 12 mm, thickness of 0.5 mm, and surface roughness of $r_a = 0.5 \pm 0.2 \mu\text{m}$. The discs were laser marked for identification, cleaned, packaged according to the typical procedures used for medical devices and sterilized by γ -irradiation with a dose of about 30 kGy.

Metal elution

The 2 different metal discs (CoCrMo alloy and stainless steel) were put into flat-bottomed, polystyrene 24-well culture plates (culture plates from

Corning Incorporated Costar[®], New York, NY, USA) and incubated for 2 days in artificial sweat (AS) (0.5% NaCl, 0.1% urea, 0.1% lactic acid, pH 6.5 according to EN 1811), complete culture medium (10% AB-serum containing RPMI-1640 medium supplemented with HEPES 25 mM (Biochrom KG Seromed, Berlin, Germany), L-glutamine (Biochrom KG Seromed), antibiotic–antimycotic solution (Gibco, Carlsbad, CA, USA), non essential amino acids (NEAA) and minimal essential medium vitamins (Biochrom KG Seromed), or distilled water. The elution time of 2 days was chosen in analogy to the 2 days of epicutaneous application during patch test. The metal discs (uncovered surface, 0.245 cm²) were exposed to 1 ml elution medium over 2 days at 37° celsius under sterile conditions. The eluates were obtained in quadruplicate, e.g. in 4 separate experimental series. The eluates were then pooled, and the metal content was assessed by a graphite furnace atomic absorption spectrometer (AAS).

Atomic absorption spectrometry

Determination of Ni, Co, and Cr in the pooled metal disc eluates was performed by graphite furnace AAS with Zeemann effect background correction system (SIMAA 6100, THGA Graphite Furnace, autosampler AS 800; Perkin Elmer, Rodgau, Germany) after microwave digestion of the samples. Transversely heated, pyrolytically coated graphite tubes with integrated L'vov platforms were used. Results were obtained from a 3-point calibration curve (5, 25, and 50 $\mu\text{g Ni/l}$) prepared from Ni standard solution (nickel atomic absorption standard solution; Sigma Chemical Company, St Louis, MO, USA) with diluted nitric acid. The same method was used

Table 1. Percentage of the main alloying elements in the stainless steel and CoCrMo alloy discs tested here and the corresponding values given by the ISO standard

Materials	Metal content (wt %)								Further elements <1.0%
	Cobalt	Chromium	Nickel	Molybdenum	Iron	Copper	Zinc	Manganese	
CoCrMo alloy test sample	65.20	27.3	0.3	5.4	0.23	n.d.	n.d.	0.8	
CoCrMo ISO standard (ISO 5832–12)	Balance	26.0–30.0	1.0 maximum	5.0–7.0	0.75 maximum			1.0 maximum	Si, C, and N
Stainless steel test sample	n.d.	20.79	9.81	2.22	62.7	0.07		4.07	
Nitrogen-alloyed stainless steel ISO standard (ISO 5832–9)		19.5–2.0	9.0–11.0	2.0–3.0	Balance	0.25 maximum		2.0–4.25	Si, C, N, Nb, S, P, and Cu

n.d., not detectable; wt%, weight percent.

for the assessment of Cr and Co. 4 independent measurements of the eluates were performed, and the mean metal content is given in $\mu\text{g}/\text{cm}^2/2$ days. For each set of tests, a blank element solution had been included in AAS measurement, and these results were subtracted from the samples. Detection limits were $0.00007 \mu\text{g}/\text{ml}$ for Ni, $0.00015 \mu\text{g}/\text{ml}$ for Co, and $0.00035 \mu\text{g}/\text{ml}$ for Cr.

Patients and patch testing

A consecutive series of 52 individuals (41 women, age 22–79 years; 11 men, age 27–65 years) who were diagnosed by history and patch testing as being metal-allergic and 48 non-allergic patients (no history of metal allergy, no patch test reactivity to Ni, Co, or Cr; 27 women, age 21–78 years; 21 men, age 21–73 years) volunteered upon informed consent to getting additionally patch tested to the metal discs (CoCrMo and stainless steel). This procedure was approved by the local ethics committee. 41 of the metal-allergic individuals were Ni allergic, 16 Co allergic, and 12 Cr allergic. 15 of the 52 were allergic to more than 1 metal (Fig. 1). The discs were fixed on the patients back and, identically to routine patch test, removed after 2 D. Evaluation of the skin reactions were performed upon removal (2 D application) and at 3 D. Reactions were classified according to the standard International Contact Dermatitis Research Group criteria.

Results

Metal release

No Co release was detectable from stainless steel after 2-day elution. The results are given in Table 2. There was only low liberation of Ni and Cr in the elution media from stainless steel – Ni: $0.30\text{--}0.46 \mu\text{g}/\text{cm}^2/2$ days and Cr: $0.06\text{--}0.38 \mu\text{g}/\text{cm}^2/2$ days. Similarly, only small amounts of Ni and Cr were also eluted from the CoCrMo alloy in the different elution media (overall Ni: up to $0.33 \mu\text{g}/\text{cm}^2/2$ days and Cr: $0.52\text{--}1.36 \mu\text{g}/\text{cm}^2/2$ days).

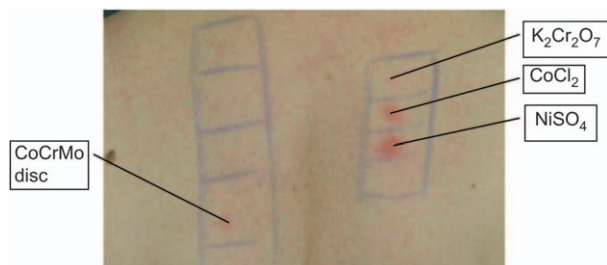


Fig. 1. Eczematous reaction to CoCrMo disc in a metal-allergic (Ni and Co) patient.

$\text{cm}^2/2$ days). There was a marked Co release from the CoCrMo alloy in the different elution media, with the highest liberation in AS as the most harsh condition (maximum $18.94 \mu\text{g}/\text{cm}^2/2$ days).

Patch testing

None of the 52 metal-allergic individuals – of whom 41 were Ni allergic – showed contact allergic reactions to the stainless steel discs. 5 of the 52 metal-allergic individuals reacted to the CoCrMo alloy disc. These 5 reactors had a particular ‘allergic profile’ with multiple contact allergies. All of them had a concomitant allergic reaction both to the CoCrMo alloy disc and to Co standard patch test. The reactions to the disc were + in 4 individuals, and 1 person showed a ++ reaction to it. The reaction to the Co standard test was + in 1, ++ in 3, and +++ in 1 individual of the 5 reactors. Thus, 5 of the 16 Co-allergic patients (31%) within the 52 metal-allergic individuals had reacted to the CoCrMo alloy disc. 3 of these 5 reactors were in addition allergic to Ni (Fig. 1) and 3 to Cr. The 48 non-allergic individuals did not react to the discs.

Discussion

Stainless steel and CoCrMo-based alloys are widely used in medical products, particularly implant materials. Upon moistening or implantation, they may release metal ions, e.g. corrode. We thus examined in a patch test model if this release of the different metals from CoCrMo disc or stainless steel disc was sufficient to elicit an allergic skin test reaction in patients with a positive patch test to Ni, Cr, or Co. In addition, we compared the results of skin testing with the release rates of respective metals as assessed by AAS of eluates from the metal discs. Patch testing to the stainless steel disc (in our case, a nitrogen-alloyed stainless steel according to ISO 5832–9) gave no positive allergic reaction in all of the 52 individuals tested, of whom 41 were Ni allergic. This is also reflected by the low release of Ni (maximally $0.46 \mu\text{g}/\text{cm}^2/2$ days upon exposure to distilled water) in the elution experiments. Our patch test results are in accordance with a previous investigation, showing patch test reactivity to a stainless steel material only in 2 of 66 individuals (15). 5 of our 52 metal-allergic patients tested with the CoCrMo disc showed an eczematous reaction to it. All these 5 reactors were allergic to Co (5/16 or 31%). Of the 5 Co-allergic patients reacting to CoCrMo disc, 3 were also allergic to Ni and 3 were also to Cr. In the elution experiments, CoCrMo discs showed substantial Cr and a marked Co release.

Table 2. Release of metal ions from stainless steel discs and CoCrMo discs by exposure to different elution media (cell culture medium, distilled water, and AS) over 2 days. Metal release was assessed by atomic absorption spectrometry. The basic metal content in the elution media was subtracted. Mean of 4 measurements is given in $\mu\text{g}/\text{cm}^2/2$ days

Eluent	Metal release from stainless steel ($\mu\text{g}/\text{cm}^2/2$ days)			Metal release from CoCrMo alloy ($\mu\text{g}/\text{cm}^2/2$ days)		
	Ni	Co	Cr	Ni	Co	Cr
Culture medium	0.35 ± 0.12	n.d. ± 0	0.06 ± 0.01	0.33 ± 0.07	13.20 ± 0.14	1.10 ± 0.01
Distilled water	0.46 ± 0.03	n.d. ± 0	0.24 ± 0.05	n.d. ± 0	16.90 ± 0.54	0.52 ± 0.02
AS	0.30 ± 0.04	n.d. ± 0	0.38 ± 0.02	n.d. ± 0	18.94 ± 0.75	1.36 ± 0.11

AS, artificial sweat; n.d., not detectable; SD, standard deviation.

Taken together, the results indicate that particularly the degree of corrosion from CoCrMo alloy is sufficient to elicit a cutaneous reaction in about one third of the patients with Co allergy. Furthermore, there is also coincidence with abundant Co release from the respective discs. Thus, in this study, we conclude that the (cutaneous) threshold level for triggering contact allergic reactions was reached.

We performed a 2-day elution of the 2 materials to have a time span comparable to the 2-day epicutaneous application of the discs. The degree of metal ion release did also depend on the type of elution media. With regard to Ni ion release, both the CoCrMo alloy (maximum, $0.33 \mu\text{g}/\text{cm}^2/2$ days to culture medium) and the stainless steel (maximum, $0.46 \mu\text{g}/\text{cm}^2/2$ days to distilled water) showed only moderate Ni liberation compared with background level in the elution media (Table 2). With regard to metal ion release *in vivo*, several investigations reported elevated levels of Ni, Co, and Cr in patients with metal-to-metal arthroplasty. It was also reported that the presence of proteins increase the corrosion of stainless steel and pure metals (16). We observed a similar effect upon use of AB-serum containing medium as eluent. The amount of Ni liberated from stainless steel may also depend on manufacturing aspects. Haudrechy et al. reported that non-resulfurized stainless steels with a very low sulfur content (for example about 0.002% in American Iron and Steel Institute AISI 316L stainless steel) did not elicit patch test reactions, whereas patch test reactivity was seen in 14% of the patients to a high-sulfur stainless AISI 303 steel and even in 96% to nickel-plated samples (17). Correspondingly, it was found that low-sulfur stainless steel does release only small amounts of Ni in AS. Furthermore, it was concluded, that nickel-plated alloys may have a much less stable 'passive film' compared with the iron–chromium oxide film covering stainless steel materials (17). In a subsequent study, it was shown that also mid-sulfur stainless steel grades released less than $0.5 \mu\text{g}/\text{cm}^2/\text{week}$ Ni as well in acidic AS as in neutral (18). In the same study,

high-sulfur stainless steel (AISI 303) led to nickel dermatitis in 4% of the Ni-allergic individuals (18).

As metals are frequent elicitors of contact allergy in the general population (1, 19, 20), potential threshold levels for elicitation of cutaneous DTH have been investigated by evaluation of metal release from and patch test reactivity to Ni-containing discs (15, 21) and chromated discs (22). The heterogeneous experimental settings in the various studies led, however, to only in part comparable results. The respective threshold levels were reported for Ni $1 \mu\text{g}/\text{cm}^2/\text{week}$ and for Cr $0.3 \mu\text{g}/\text{cm}^2$ after short time 20-min elution. In the case of Co, serial dilutions of CoCl_2 in distilled H_2O were used for patch test in 9 Co-allergic individuals, and the minimal DTH eliciting concentration was reported as 2.26 ng/ml (23). Data regarding CoCrMo-based materials were missing until now. Furthermore, modifying factors, like closed or penetrating exposure, single or repeated application, presence of an irritant or irritated skin, vehicle used or study criteria, may change clinical reactivity (18, 24). Using a finger immersion method (25, 26) and by a coin-handling experiment (27), additional assays were applied to understand the role of short-time repetitive metal (nickel) contact. There surely exist strongly sensitized individuals in whom minute exposure to metals may already provoke skin eruptions (28) and also lead to unusual clinical manifestations (29–31). In general, however, as alloys with a Ni release more than $1 \mu\text{g}/\text{cm}^2/\text{week}$ were found to produce strong eczematous, allergic reactions in Ni-sensitive individuals (15), it was suggested to use alloys with a Ni release rate of less than $0.5 \mu\text{g}/\text{cm}^2/\text{week}$ for metal items designed for prolonged skin contact. This recommendation was enforced by the 'Nickel Directive' of the European Parliament (32). Respective recommendations do not exist for Co-containing materials. The results of the present study suggest that the role of Co as contact allergen may be underestimated particularly upon its use in CoCrMo alloys.

In summary, to date, elicitation thresholds for cutaneous metal-allergic reactions have been studied mostly for Ni contact allergy. Based on the present results also, Co release from CoCrMo alloys is sufficient to elicit a positive cutaneous reaction. Because of difficult experimental approach, characterization of such allergy elicitation thresholds in peri-implant tissue is largely missing. In view of our findings, the role of Co in hyperre-activity reactions to arthroplasty may have been underestimated so far.

Acknowledgement

This study was supported by a grant of AESCULAP, Tuttlingen.

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