

Cross-reactivity between nickel and palladium demonstrated by systemic administration of nickel

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Concomitant patch test reactions to nickel and palladium have frequently been reported in patients undergoing investigation because of suspected allergic contact dermatitis. Theoretically, these reactions can be explained by multiple, concomitant, simultaneous sensitization as well as cross-sensitization. We studied whether concomitant reactions to nickel and palladium could represent cross-sensitization in females hypersensitive to combinations of nickel, palladium and cobalt. Females were patch tested with serial dilutions of nickel sulfate, cobalt chloride and palladium chloride on the upper back. 1 month later, when the patch test reactions were gone, the patients were randomized into 2 groups that were challenged orally with either nickel or placebo. 1 day later, the areas of previous positive patch test reactions were read in a blind way looking for flare-up reactions. Nickel provocation but not placebo yielded flare-up reactions on sites previously tested with nickel ($P = 0.012$) and palladium ($P = 0.006$), but were also observed on sites previously tested with cobalt, even though this was not statistically significant. Flare-up reactions of previous patch test reactions to nickel and palladium after oral challenge with nickel speak in favour of a cross-reactivity mechanism.

Key words: atomic absorption spectrometry; chemical analysis; cobalt; cross-reactivity; nickel; oral challenge; palladium. © Blackwell Munksgaard, 2005.

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The use of palladium has increased in the past 10 years (1). Palladium is used in dental appliances, jewellery, chemical catalysts, electrical appliances and as an automotive emission-control catalyst (1). The general population may be exposed to palladium mainly through dental alloys and jewellery. The use of palladium–silver alloys has, since their introduction in 1973, increased significantly and they now comprise a substantial part of the noble metal ceramic alloy sales in the United States (2).

However, palladium is, like nickel, a known sensitizer in humans and guinea pigs (3), and simultaneous patch test reactions to nickel and palladium have frequently been reported (1, 4–10). Contamination of palladium and nickel with each other has already been excluded as the cause of simultaneous allergies (5, 11). Multiple, concomitant and simultaneous sensitization as well as cross-sensitization (12) are possible theoretical explanations for the association between nickel and palladium allergy. Nickel and palladium belong to the same group in the periodic table, which suggests cross-sensitization,

an interpretation also supported by *in vitro* studies. Palladium hypersensitivity in nickel-allergic patients may be due to cross-reactivity at the T-cell clonal level (13, 14). In one study, simultaneous reactions to nickel and palladium were considered to depend on the generation of similar complexes between the ions and the skin proteins and not the metal itself (15).

In a study with guinea pigs, palladium chloride was found to be a more potent sensitizer than nickel sulfate (3).

The present study was designed to investigate the nature of simultaneous contact allergies to nickel and palladium by using a model with systemic hapten challenge occasioning specific flare-up reactions of previously positive patch test sites.

Materials and Methods

Subjects

27 females (mean age 36.4 years, range 19–60 years) were enrolled in the study. These females belonged to 3 different groups with regard to the

presence of contact allergy to the metals nickel, cobalt and palladium: (i) group A, 5 females hypersensitive to nickel and cobalt; (ii) group B, 18 females hypersensitive to nickel, cobalt and palladium; and (iii) group C, 4 females hypersensitive to palladium. The hypersensitivity had been demonstrated by patch testing 0.5–5 years prior to the study with the European standard series, supplemented with palladium chloride at 2.0% w/w in petrolatum. The females were enrolled in the study after approval by the Ethics Committee of Lund University, Medical Faculty. Informed consent was obtained from each patient.

Substances

Nickel(II)sulfate hexahydrate, cobalt(II)chloride hexahydrate and palladium chloride were purchased from Acros Organics (Geel, Belgium) and INC Biomedicals (Aurora, OH, USA), respectively.

Patch testing

All females were patch tested with serial dilutions of the metal salts of nickel, cobalt and palladium. An aqueous stock solution of nickel at 12.5% w/v was prepared and further diluted by a factor of 2.5 down to 0.0032%. An aqueous stock solution of cobalt at 7.8% w/v was prepared and further diluted by a factor of 2.5 down to 0.0021%. Palladium at 5.0% w/w in petrolatum was prepared and diluted by a factor of 2.5 down to 0.05%. Petrolatum was used as vehicle as palladium chloride was virtually insoluble at the desired concentration in suitable solvents for patch testing.

15 µl of the nickel and cobalt test solutions was micropipetted onto filter paper discs of small Finn Chambers® (Epitest Ltd Oy, Tuusula, Finland) on Scanpor tape (Norgesplaster A/S, Vennesla, Norway). The palladium preparations were also applied (approximately 25 mg) onto Finn Chambers® on Scanpor tape. The tests were applied on the upper part of the back in a randomized order for the respective dilution series and remained there for 2 days. The reading was performed on day 3, according to internationally accepted criteria.

To enable exact localization of the test area, the distance from the most proximal and distal Finn Chamber® borders to the spine was measured on each test occasion. We also measured the distance to the lowest part of scapula, hip and the most prominent vertebra of the neck.

Oral challenge

1 month after patch testing, when the test reactions were completely gone, the patients in the

group with previous hypersensitivity to nickel, cobalt and palladium (group B) were randomly divided into 2 groups. The patients were instructed not to eat or drink from midnight before attending the clinic at 8 AM. Under observation, all females in group A, 13 females in group B and all females in group C then ingested a capsule containing 13.44 mg nickel sulfate (nickel sulfate $\times$ 6H₂O) in lactulose, the nickel content being 3.0 mg. 5 females in group B ingested a placebo capsule. The patients were instructed not to eat until 1 hr after ingestion of the nickel or placebo capsules. The patients returned to the clinic 24 hr later. The patients and the test reader did not know to which group the patients belonged or the localization of the serial dilutions of the metal salts patch tested, and the reader did not know the combination of metal allergies of the patients. Flare-up of previous patch test reactions was scored according to internationally accepted criteria. Thereafter, other localized and/or systemic eczematous, oedematous and/or toxicoderma-like flare-up reactions were looked for.

Atomic absorption spectrometry

The nickel content in the nickel capsule and the contamination of cobalt and palladium in the nickel and lactulose capsules, respectively, were investigated with atomic absorption spectrometry (THGA graphite furnace, Perkin Elmer A Analyst 800, Perkin Elmer LLC, Norwalk, CT, USA). Detection limits per capsule for cobalt and palladium were below 0.05 and 0.1 µg, respectively. The nickel content in cobalt(II)chloride hexahydrate and palladium chloride used for patch testing was 0.016% w/w for the cobalt salt and below the detection limit of 0.001% w/w in the palladium salt. The content of cobalt and palladium in nickel(II)sulfate hexahydrate used for patch testing was below the respective detection limits of 0.001% and 0.002% w/w.

Statistical calculations

Fischer's exact test (2-sided) was used. For the statistical analyses, the scoring as negative or positive flare-up reaction of each patch test site was used.

Results

In group A, only 1 patient was hypersensitive to both nickel and cobalt (Tables 1 and 2), 2 more patients were allergic to cobalt alone and 1 patient to nickel alone. In group B, 14 patients were hypersensitive to all 3 metals. 4 patients did

Table 1. The results of patch testing with serial dilutions of nickel, palladium and cobalt, as well as the results of oral challenge with nickel 1 month after patch testing when the test reactions had gone

Patch test preparation	Group A					Group B					Group C											
	1*	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Nickel (%)																						
12.5	-	n	y	n	-	n	+	y	+	+	y	+	+	y	+	+	n	+	y	-	n	-
5	-	n	y	n	-	n	+	y	+	+	y	+	+	y	+	+	n	+	y	-	n	-
2	-	n	n	n	-	n	+	y	+	+	y	+	+	y	+	+	n	+	y	-	n	-
0.8	-	n	n	n	-	n	+	y	+	+	y	+	+	y	+	+	n	+	y	-	n	-
0.32	-	n	n	n	-	n	+	y	+	+	y	+	+	y	+	+	n	+	y	-	n	-
0.13	-	n	n	n	-	n	+	y	+	+	y	+	+	y	+	+	n	+	y	-	n	-
0.05	-	n	n	n	-	n	+	y	+	+	y	+	+	y	+	+	n	+	y	-	n	-
0.02	-	n	n	n	-	n	+	y	+	+	y	+	+	y	+	+	n	+	y	-	n	-
0.008	-	n	n	n	-	n	+	y	+	+	y	+	+	y	+	+	n	+	y	-	n	-
0.0032	-	n	n	n	-	n	+	y	+	+	y	+	+	y	+	+	n	+	y	-	n	-
Cobalt (%)																						
7.8	+++	y	+	n	+	+	+	y	+	+	n	+	+	+	+	+	n	+	+	y	-	n
3.12	+++	y	+	n	+	+	+	y	+	+	n	+	+	+	+	+	n	+	+	y	-	n
1.25	+++	y	+	n	+	+	+	y	+	+	n	+	+	+	+	+	n	+	+	y	-	n
0.5	++	y	+	n	+	+	+	y	+	+	n	+	+	+	+	+	n	+	+	y	-	n
0.2	+	n	n	n	-	n	+	y	+	+	n	+	n	+	+	+	n	+	+	y	-	n
0.08	-	n	n	n	-	n	+	y	+	+	n	+	n	+	+	+	n	+	+	y	-	n
0.032	-	n	n	n	-	n	+	y	+	+	n	+	n	+	+	+	n	+	+	y	-	n
0.013	-	n	n	n	-	n	+	y	+	+	n	+	n	+	+	+	n	+	+	y	-	n
0.0052	-	n	n	n	-	n	+	y	+	+	n	+	n	+	+	+	n	+	+	y	-	n
0.0021	-	n	n	n	-	n	+	y	+	+	n	+	n	+	+	+	n	+	+	y	-	n
Palladium (%)																						
5	-	n	n	n	+	n	+	y	+	+	y	+	+	+	+	+	n	+	y	+	+	+
2	-	n	n	n	-	n	+	y	+	+	y	+	+	+	+	+	n	+	y	+	+	+
0.8	-	n	n	n	-	n	+	y	+	+	y	+	+	+	+	+	n	+	y	+	+	+
0.32	-	n	n	n	-	n	+	y	+	+	y	+	+	+	+	+	n	+	y	+	+	+
0.13	-	n	n	n	-	n	+	y	+	+	y	+	+	+	+	+	n	+	y	+	+	+
0.05	-	n	n	n	-	n	+	y	+	+	y	+	+	+	+	+	n	+	y	+	+	+

y = yes, presence of flare-up reaction after oral provocation; n = no, absence of flare-up reaction after oral provocation.

Table 2. The results of patch testing with serial dilutions of nickel, palladium and cobalt, as well as the results of oral challenge with placebo 1 month after patch testing when the test reactions had gone

Patch test preparation Patient	Placebo (group B)				
	1	2	3	4	5
Nickel (%)					
12.5	+	n	+	+	n
5	(+)	n	(+)	+	n
2	+	+	–	+	+
0.8	+	+	–	+	–
0.32	(+)	–	–	+	–
0.13	–	–	(+)	+	n
0.05	–	–	–	–	n
0.02	–	–	–	+	n
0.008	–	–	–	–	n
0.0032	–	–	–	–	n
Cobalt (%)					
7.8	+	n	+	+	n
3.12	+	n	+	+	+
1.25	+	n	–	+	+
0.5	(+)	n	–	+	–
0.2	(+)	n	–	+	–
0.08	–	n	–	–	n
0.032	–	n	–	–	n
0.013	–	n	–	–	n
0.0052	–	n	–	–	n
0.0021	–	n	–	–	n
Palladium (%)					
5	+	n	+	+	n
2	(+)	n	+	+	+
0.8	–	n	–	+	n
0.32	–	n	–	+	n
0.13	–	n	–	–	n
0.05	–	n	–	–	n

y = yes, presence of flare-up reaction after oral provocation; n = no, absence of flare-up reaction after oral provocation.

not test positively to cobalt and 1 of them also had a negative palladium test.

In only 2 of the previous palladium-allergic individuals were the positive palladium tests repeated (group C).

Tables 1 and 2 summarize the number of flare-ups of previous patch test reactions in the different groups.

Compared to the placebo group, statistically significantly more individuals with flare-up reactions were seen at previous nickel-positive ($P = 0.012$) and palladium-positive ($P = 0.006$) patch test sites. No significant flare-up reactions were seen of previous cobalt-positive ($P = 0.09$) patch test sites. In the placebo group, no signs of flare-up reactions were observed.

Cobalt and palladium were not detected in the nickel salt used for patch testing or in the nickel and lactulose capsules. The amount of nickel in the 3 mg nickel capsule was 2980 μg (99.3%). Nickel was not detected in the palladium salt used for patch testing, while nickel was present in the cobalt salt used for patch testing at a concentration of 0.016% w/w.

The correlation between the intensity of previous patch test reactions and number of flare-up reactions is illustrated in Fig. 1. The dose-response curves for flare-up reactions of nickel and palladium on oral challenge with nickel look very similar, with a seemingly higher tendency for previous positive patch test reactions to palladium to flare as compared with previous positive nickel patch test reactions. For all 3 metals, flare-up reactions were noted at patch test sites where

the previous patch testing had resulted in negative reactions. For nickel, palladium and cobalt, 13.4%, 28.9% and 3.5%, respectively, of the previous negative patch test reactions flared on oral challenge with nickel. In 3 individuals with previous contact allergy to nickel and in 2 individuals with previous contact allergy to palladium, all patch test reactions were negative, and in these 5 individuals there were no flare-up reactions on oral nickel challenge.

Discussion

Significantly more flare-up reactions of previously positive patch test reactions to nickel and palladium were noticed after oral nickel provocation compared to placebo. On examination of the females 24 hr after the oral nickel challenge, a mixed eczematous-urticarial reaction was observed at the site of previous nickel and palladium patch tests, as well as at sites where previous allergic contact dermatitis from nickel had been localized. However, although grading of the positive reactions was carried out, only the classification into positive and negative flare-up reactions of previous patch test sites was used for statistical calculation. In the 15 individuals with positive patch test reactions to nickel in this study (Tables 1 and 2), 11 individuals (73%) showed flare-up reactions on oral nickel challenge, while the same nickel challenge dose in another study (16) resulted in flare-up of all previously positive nickel patch tests. This recent study (16) also demonstrated the flare-up reactions after oral

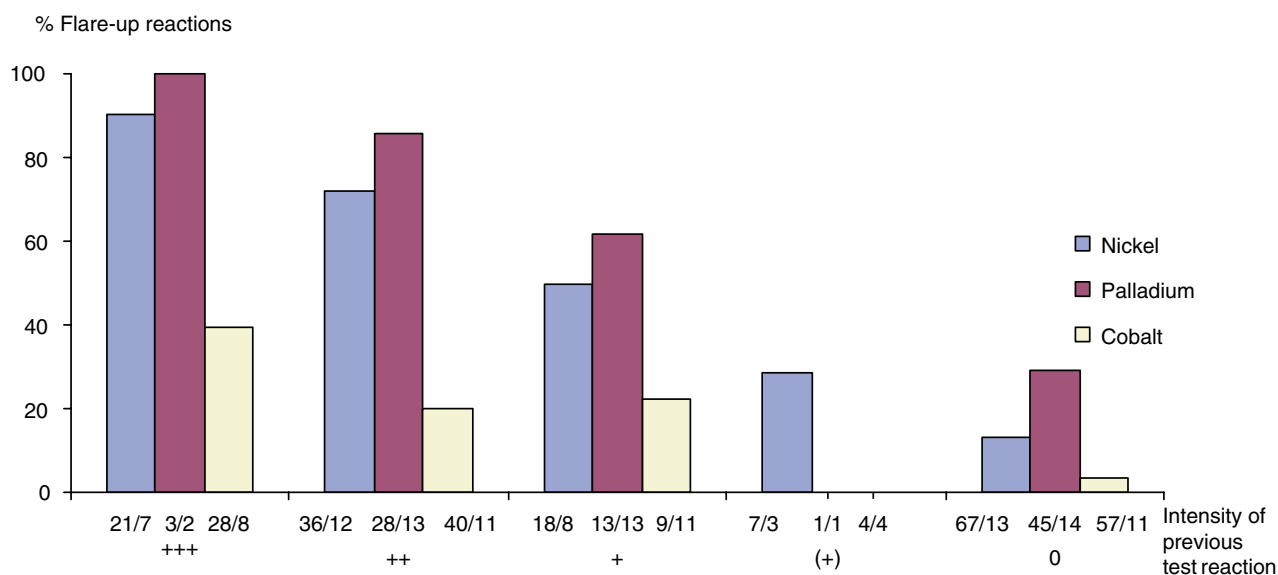


Fig. 1. The proportion (%) of previous patch test reactions which flare-up on oral nickel challenge with regard to the intensity of previous test reactions for each test substance. Below the separate groups of bars the total numbers of patch test reactions for each intensity level are given as well as the numbers of patients with at least 1 reaction of that particular reactivity (+++ bar, 21/7 = 21 +++ patch test reactions to nickel in 7 patients).

nickel challenge to be clearly dose dependent (16) and the significance of the time interval between the previous nickel reaction and the oral provocation. Similarly to the above-mentioned study (16), we found flare-up reactions at previous test sites to be clearly dependent of the intensity of previous patch test reactions, the stronger the patch test reaction the more prone to flare on oral challenge (Fig. 1). We also found as in the previous study (16), flare-up reactions where there had been no visible reaction on initial patch testing. This phenomenon was observed at previous nickel, palladium and cobalt patch test sites (Tables 1 and 2; Fig. 1). All females enrolled in the study had at least a ++ reaction to nickel sulfate, palladium chloride or cobalt chloride 0.5–5 years prior to the study. When tested this time, some of the females had completely negative patch test reactions, although the highest test concentration this time was 2.5 times greater. This variation in test reactivity has previously been shown in nickel reactivity (17, 18).

Theoretically, possible interpretations of the results of nickel provocation in this study comprise systemic reactions mediated by nickel through either an allergic or a pharmacological/toxic mechanism. Systemic contact dermatitis encompasses flare-up reactions of previous eczema sites, including vesicular hand eczema, as well as previous patch test sites, after oral challenge with the sensitizer. The flare-up reactions of previous patch test sites together with the 'Baboon syndrome' (19) are considered to be the most specific cutaneous symptoms in systemic contact dermatitis (20–22). Experimentally, flare-up reactions after oral challenge with the sensitizer have been demonstrated to be specific. In patients with contact allergy to gold, no flare-up reactions were observed when challenged with nickel and no reactions were seen when patients allergic to nickel were challenged with gold (23). Furthermore, systemic gold and nickel provocations did not result in any reactions in the controls (23). Neither has oral nickel challenge in nickel-hypersensitive persons caused flare-up reactions on previous irritant eczema sites (24). Therefore, with this background, flare-up reactions of previous palladium patch test sites on oral nickel provocation in this study are strong evidence of cross-sensitization, particularly as no nickel contamination was demonstrated in the palladium salt used for patch testing. Interestingly, the same number of individuals (10 of 13) with positive patch test reactions to both nickel and palladium, showed flare-up reactions on oral challenge with nickel (Tables 1 and 2). Furthermore, the correlation between the

intensity of previous patch test reactions and number of flare-up reactions was similar for nickel and palladium, although there was a tendency for a greater flare-up reactivity for palladium (Fig. 1). In this context, patient no. 16 (Table 1) seems to be a currently unexplained extreme reactor, with positive patch test reactions to all metals but flare-up reactions only to palladium after oral nickel provocation. Initially, we planned to carry out the same study with patch testing with serial dilutions with nickel, palladium and cobalt followed by systemic administration of palladium. However, due to the possible general toxicity of systemic palladium, we refrained from this experiment, even though palladium has previously been given in the treatment of tuberculosis (25).

To our surprise, we also found flare-up reactions of previous cobalt patch test reactions, even though this was not statistically significant. Simultaneous patch test reactions to cobalt and nickel, as for nickel and palladium, have frequently been reported (7, 9, 10). However, in this study, cobalt was chosen a negative control because simultaneous contact allergies to cobalt and nickel have been considered to be explained by mechanisms other than cross-reactivity (12, 26, 27). Theoretically, nickel contamination of the cobalt salt used for patch testing could explain the flare-up reactions after oral nickel provocation in the present study, but chemical analysis demonstrated only insignificant and negligible nickel contamination. These cobalt flare-up reactions after systemic nickel provocation should therefore be subjected to further investigation to see whether they represent a reproducible phenomenon. Theoretically, systemic and topical exposures to haptens may give rise to different antigens with different cross-reaction patterns.

In conclusion, the results of this study speak strongly in favour of cross-reactivity between nickel and palladium.

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