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Cross-reactivity to Metal Compounds Studied in Guinea Pigs Induced with Chromate or Cobalt

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Multiple sensitivity to metals is often seen in humans. This may be due to cross-reactivity or to multiple sensitization. The pattern of cross-reactivity to some metal compounds was studied, using the guinea pig maximization test method. Animals were induced with $K_2Cr_2O_7$ and $CoCl_2$, respectively, and then challenged with $K_2Cr_2O_7$, $CoCl_2$, $NiSO_4$, $PdCl_2$ and $RhCl_3$. No simultaneous reactivity was recorded. This supports the view that simultaneous reactivity in patients to nickel-cobalt and to chromate-cobalt is due to multiple exposure and sensitization, and not to cross-reactivity. The purity of the test compounds was analysed. ***Key words: contact allergy; guinea pig maximization test; nickel; palladium; rhodium; purity.***

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Some metals belong to the most frequent contact sensitizers in humans, and quite often multiple sensitivity to metals is seen. Some of these reactions are said to be due to multiple sensitization and others to cross-reactivity. In previous studies we have shown that $PdCl_2$ and $RhCl_3$ are potent sensitizers and that true cross-reactivity to $NiSO_4$ is induced in guinea pigs sensitized to $PdCl_2$, and to $CoCl_2$ in guinea pigs sensitized to $RhCl_3$ (1–3). The possible cross-reactivity to the well-known contact allergens nickel, chromate and cobalt has not, however, been examined in animal models. The cross-reactivity pattern is essential for advice concerning the prevention of relapses of allergic contact dermatitis.

The aim of the present study was to investigate the pattern of cross-reactivity to some metal compounds. Animals induced with chromate and cobalt, respectively, were challenged with salts of 5 different metals. The purity of the test compounds was checked using inductive coupled plasma detection.

MATERIALS AND METHODS

Chemicals

The following metal compounds were used: potassium dichromate ($K_2Cr_2O_7$), p.a.; cobalt chloride ($CoCl_2 \cdot 6H_2O$), p.a.; and nickel sulphate ($NiSO_4 \cdot 6H_2O$), p.a., all from Merck, Darmstadt, Germany; palladium chloride ($PdCl_2$), 59.84% Pd; and rhodium chloride ($RhCl_3$), 40.42% Rh, from Johnson Matthey, Materials Technology, UK.

Freund's complete adjuvant (FCA) from Difco Laboratories, Detroit, Mich., USA and white petrolatum (pet.) of pharmaceutical quality, produced by Apoteksbolaget AB, Göteborg, Sweden, were used.

Chemical analysis

Inductive coupled plasma detection (ICP) was used to analyse the concentration of contaminants in the metal salts used for induction and challenge. ICP is a sensitive analytical method, useful for the analysis of several elements simultaneously. Very high temperature causes emission from the elements. The emission is detected by a spectrometer (4). The analyses were carried out by Analytica AB, Täby, Sweden.

Animals

The animals used were female Dunkin-Hartley guinea pigs from AB Sahlins Försöksdjursfarm, Malmö, Sweden. The average weight was 300 g at induction.

Sensitization method

The guinea pig maximization test (GPMT) method was used, as described in a previous paper (5).

Concentrations for induction and challenge were determined in guinea pigs pretreated with FCA. For intradermal induction the slightly irritant concentrations of 0.5% $K_2Cr_2O_7$ and 1% $CoCl_2$, in water, were chosen. One percent $K_2Cr_2O_7$ and 5% $CoCl_2$, in pet., were chosen for topical induction.

The guinea pigs were divided into 3 groups with 15 animals in each.

Table I. *Induction with $K_2Cr_2O_7$ and $CoCl_2$, respectively, in the GMPT*

Challenge with various metal salts in pet. and with pet. (vehicle control). The number of pos. animals at the 48 h reading is given

Challenge substance, conc. (%)	$K_2Cr_2O_7$	$CoCl_2$		$NiSO_4$		$PdCl_2$	$RhCl_3$	Control, pet.
	0.3	0.3	0.1	1	0.3	1	3	100
Group I Induced with $K_2Cr_2O_7$ ($n=15$)	8[a]	1[b]	1[c]	0	0	0	0	1
Group II Induced with $CoCl_2$ ($n=15$)	0	11[a]	5	1[d]	0	0	0	0
Group III Sham-treated controls (n = 15)	0	1	2	0	1	1	1	1

[a] $p<0.001$ compared to the control group.
[b] This animal was also pos. to $K_2Cr_2O_7$.
[c] This animal was neg. to $K_2Cr_2O_7$.
[d] This animal was also pos. to $CoCl_2$ 0.3%.

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Table II. *Analysis with inductive coupled plasma detection (ICP) of metal contaminants in metal salts used for induction and challenge in the GPMT*

– = main component, not analysed with ICP; n.a. = not analysed

Sample	Contaminants (ppm)				
	Cr	Co	Ni	Pd	Rh
$K_2Cr_2O_7$	–	< 1.0	< 0.5	< 100	n.a.
$CoCl_2$	n.a.	–	n.a.	n.a.	5.1
$NiSO_4$	n.a.	n.a.	–	< 1.0	n.a.
$PdCl_2$	n.a.	n.a.	< 20	–	n.a.
$RhCl_3$	< 0.25	< 0.5	< 0.25	< 5.0	–

Group I was induced with $K_2Cr_2O_7$, and group II with $CoCl_2$. Group III was a sham-treated control group.

Challenge

For challenge, non-irritant concentrations of $K_2Cr_2O_7$, $CoCl_2$, $NiSO_4$, $PdCl_2$ and $RhCl_3$, all in pet. (Table I), were chosen, based on previous findings in our laboratory. Closed challenge with Finn chambers (Epitest, Helsinki, Finland) was used. All animals were challenged in one session with all preparations, as shown in Table I. Vehicle controls, rotation of test sites, and blind readings were used at challenge. The minimum criterion for a positive reaction was a confluent erythema.

Statistical analysis

Statistical analysis was carried out by χ^2 analysis or Fisher's exact test.

RESULTS

The results from the GPMT are shown in Table I. Eight out of 15 animals induced with $K_2Cr_2O_7$ became sensitized to $K_2Cr_2O_7$, and 11/15 animals induced with $CoCl_2$ became sensitized to $CoCl_2$, as shown by the challenge. No simultaneous reactivity to the other metal salts used at challenge was recorded.

The results from the chemical analyses are shown in Table II.

DISCUSSION

$K_2Cr_2O_7$ and $CoCl_2$ were shown to be potent sensitizers in the guinea pig. This has been shown previously (6, 7). The animals did not react to the other metal salts used at challenge (Table I), however, and to the best of our knowledge this has not been demonstrated before. Thus, no indication of cross-reactivity in chromate- or cobalt-sensitive animals was found.

Our findings support the view that the pattern of multiple metal sensitivity to nickel-cobalt and to chromate-cobalt in humans is due to multiple exposure and sensitization, and not to cross-reactivity (8, 9). The question of possible cross-reactivity to substances to which exposure is common, such as nickel, chromium and cobalt, is hard to evaluate in humans. Guinea-pig studies, however, offer the opportunity to study cross-reactivity by induction with single allergens. Challenge in *one* session with all substances of interest, as in the present study, is preferable, to avoid confounding by possible secondary sensitization.

When discussing simultaneous patch test reactions, cross-reactivity and multiple sensitization, one has to evaluate the purity of the chemicals used, so as to rule out the possibility that contaminants may be the cause of the reactivity (10, 11). In the present study, however, no multiple reactivity was recorded. The chemical analyses showed low levels of contaminants of interest (Table II).

In similar studies in guinea pigs at our department, multiple reactions were interpreted as a sign of cross-reactivity, i.e. induction with $PdCl_2$ and positive challenge with both $PdCl_2$ and $NiSO_4$ (1, 2), and induction with $RhCl_3$ and positive challenge with both $RhCl_3$ and $CoCl_2$ (3).

The pattern of simultaneous reactivity to metal salts shown in guinea pigs indicates that cross-reactivity among the transition metals may be restricted to groups but not to periods in the periodic table of elements (see Fig. 1). Chromium, cobalt and nickel belong to the same period, and no cross-reactivity was shown in guinea pigs (Table I). On the other hand, cobalt and rhodium belong to the same group and nickel and palladium belong to another group of the periodic table. This hypothesis has to be tested in further experiments.

Information on the clinical relevance of multiple metal sensitivity, and possible cross-reactivity, is limited. It is important to gain more knowledge so that adequate advice concerning primary prevention can be given to authorities and industry as to which metals should be avoided or restricted in products to limit the risk of sensitization (12). The use of nickel in jewellery and associated products will probably soon be restricted by the EU (13). This may lead the industry to increase the use of cobalt, which has similar technical properties (14). This in turn may cause an increase in cobalt sensitization, since $CoCl_2$ is a potent sensitizer in guinea pigs and also i humans. Our knowledge of the sources of cobalt sensitization in humans is insufficient. Should an increase in cobalt exposure be avoided? Palladium, which is a rather cheap precious metal, is frequently used in jewellery and dental alloys. The clinical relevance of contact allergy to palladium is, however, only slightly known. Contact allergy to $PdCl_2$ is seen in nickel-sensitive patients, and few cases are reported with a solitary allergy (15, 16). Increased knowledge concerning cross-reactivity to metals is also essential for secondary prevention: what metal exposure should be avoided by persons who are already sensitized to one or more metals? The answers to these types of questions will affect the attitude towards metal exposure at work and in leisure time, and possibly also exposure to orthopedic and dental materials.

We have developed an animal model (17), where guinea pigs sensitive to cobalt are treated with $CoCl_2$ in different concentra-

24 **Cr** **+2,+3,+6**	25 Mn +2,+3,+4,+7	26 Fe +2 +3	**27** **Co** **+2,+3**	**28** **Ni** **+2,+3**	29 Cu +1,+2	30 Zn +2
42 Mo +6	43 Tc +4,+6,+7	44 Ru +3	**45** **Rh** **+3**	**46** **Pd** **+2,+4**	47 Ag +1	48 Cd +2
74 W +6	75 Re +4,+6,+7	76 Os +3,+4	77 Ir +3,+4	78 Pt +2,+4	79 Au +1,+3	80 Hg +1,+2

Fig. 1. Some of the transition elements in the periodic table of the elements. Atomic symbol, atomic number and oxidation states are given (from the Merck Index).

tions to mimic the repeated open application test (ROAT) in humans. This model could also be used for further studies on cross-reactivity, i.e. in animals treated with different metal compounds.

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