

Cross-reactivity patterns of palladium and nickel studied by repeated open applications (ROATs) to the skin of guinea pigs

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Cross-reactivity is usually studied with patch test techniques, but the relevance of a single 1–2 day exposure under occlusion can be questioned. To study relevance, animals were induced with PdCl₂ or NiSO₄ according to the guinea pig maximization test method and then treated for 10 days according to the repeated open application test (ROAT) method. Animals induced with PdCl₂ reacted in the ROATs to PdCl₂ (100%) but rarely to NiSO₄. Animals induced with NiSO₄ reacted in ROATs to the same degree with NiSO₄ and PdCl₂ (23–30%). The concordance between pre-ROAT patch test results and ROAT outcome was high for PdCl₂ (100%) and low (10–40%) for NiSO₄. Patch testing seems to overestimate the risk of skin reactions when guinea pigs sensitive to PdCl₂ are treated topically with NiSO₄. The finding from patch test studies that animals induced with NiSO₄ react only to NiSO₄ but not to PdCl₂ was not confirmed. Repeated open applications more adequately mimic exposure conditions than does patch testing.

Key words: contact allergy; guinea pig maximization test; nickel sulfate; palladium chloride; patch testing; relevance; cross-reactivity. © Munksgaard, 1999.

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There have been several reports recently on concomitant patch test reactivity to palladium and nickel compounds. Of subjects with palladium allergy, 90.6–100% also show positive reactions to nickel sulfate (NiSO₄) (1–6). Of 11,516 tested eczema patients, 23% reacted to NiSO₄ and 8% to palladium chloride (PdCl₂) (7). In a panel of human nickel-reactive T-lymphocyte clones, 6 out of 20 showed in vitro cross-reactivity to PdCl₂ (8). The relationship between reactivity to salts of palladium and nickel is intriguing, and several explanations of the association of palladium and nickel allergy have been suggested, as reviewed by Todd & Burrows (2). Most authors favour cross-reactivity. Possibly the explanation is that palladium and nickel belong to the same group in the periodic table, but recent stereochemical analyses have provided a supplementary explanation; the formation of an octahedral structure of both nickel and palladium salts (5). Cross-reactivity is usually studied by simultaneous patch-test application of an allergen and possibly cross-reacting compounds (9) and

more recently by the re-test model (10). Purity of test chemicals is crucial (11).

In a previous study in guinea pigs, we found that animals induced with PdCl₂ reacted to PdCl₂ as well as to NiSO₄ on patch testing (day (D) 24) (12). On the other hand, animals induced with NiSO₄ reacted to NiSO₄ but not to PdCl₂.

The clinical relevance of patch test reactivity to chemicals at cross-challenge is hard to state, but it is usually implied that the patient might risk a relapse of contact dermatitis when exposed to the cross-reacting compound(s). How clinically relevant is a concomitant positive patch test reaction obtained after a single D1–2 exposure? The repeated open application test (ROAT), introduced in 1986 (13), is increasingly used and its value in studying relevance has been verified in a number of papers (14, 15).

The present aim was to study relevance – using ROAT – by applying the inducing allergen (PdCl₂ or NiSO₄) as well as the possibly cross-reacting compound (NiSO₄ or PdCl₂) topically in guinea

pigs. Sensitivity thresholds were determined pre-ROAT and post-ROAT by serial dilution tests.

Materials and Methods

Chemicals

The following chemicals were used:

Palladium chloride (PdCl_2), water free for synthesis, and nickel sulfate ($\text{NiSO}_4 \cdot 6 \text{H}_2\text{O}$) p.a. from E Merck, Darmstadt, Germany. When purity was checked, no nickel was detected in the PdCl_2 (detection limit 2 ppm by atomic absorption spectrometry with furnace) and no palladium in the NiSO_4 (detection limit 1 ppm by inductively coupled plasma detection, ICP) (by Analytica AB, Täby, Sweden).

Freund's complete adjuvant (FCA) from Difco, Detroit, Michigan, USA.

Dimethylsulfoxide (DMSO) from E Merck, Darmstadt, Germany.

Petrolatum (pet.) from Apoteksbolaget, Stockholm, Sweden.

Experimental animals

Female albino guinea pigs of the Dunkin-Hartley strain (Sahlin, Malmö, Sweden) were used. The average weight was 300 g when induction began. The study was approved by the local ethical committee.

Induction of contact allergy (Table 1)

The guinea pig maximization test (GPMT) method was used according to the same protocol as in previous studies (11, 12, 16). PdCl_2 0.03% (w/w) in saline and in FCA was used for intradermal induction on D0, and the slightly irritant concentration 2.5% in saline for topical induction on D7. NiSO_4 2.0% (w/w) in saline and in FCA was used for intradermal induction on D0, and the slightly irritant concentration 5.0% pet. for topical induction. Control animals were treated in the same way (FCA, saline, pet., occlusion with Elastoplast®, etc.) as in the experimental groups, except that the allergen was omitted.

Table 1. Experimental outline

D0 and 7	induction of contact allergy with PdCl_2 or NiSO_4 (GPMT)
D21	pre-ROAT patch testing with the inducing allergen to define sensitivity thresholds
D35–44	ROATs for 10 days with the inducing allergen, with the possibly cross-reacting chemical (in 3 concentrations) and with the vehicle
D60	post-ROAT patch testing with the inducing allergen (2 concentrations) and with the possibly cross-reacting chemical (3 concentrations)

Patch testing

Pre-ROAT. The induced guinea pigs were patch tested (serial dilution tests) using Finn Chambers (diameter 8 mm, Epitest Ltd, Hyrylä, Finland) on the clipped flanks on D21. The non-irritant concentrations used for animals induced with PdCl_2 were: 2.5, 1.25, 0.625, 0.313, 0.156%, and a vehicle control (saline). For NiSO_4 the concentrations were 1.0, 0.5, 0.25, 0.125, 0.067%, and a vehicle control (pet.).

Post-ROAT. 2 concentrations of the allergen used for induction, 3 concentrations of the possibly cross-reacting allergen, and a vehicle control, were applied.

Reading

Rotation of test sites (3 per flank) and blind reading were used. The chambers were removed at D1 and the test sites were read at D2 and D3 after application, according to a 4-grade scale ranging from 0 to +++: 0=no visible reaction; +=patchy erythema; ++=confluent erythema; +++=erythema and oedema (17).

Topical treatment (ROAT)

3 circular areas (diameter 30 mm) on the back were demarcated with ink. 1 site was treated with the allergen used for induction, 1 with the possibly cross-reacting chemical and the 3rd with the vehicle. The sites were clipped prior to reading and treatments. These were carried out once daily on D35–44 (treatment D0–9) and reading also on D45.

100 μl of PdCl_2 (1.0, 0.1, or 0.01%) in saline, or NiSO_4 (0.1, 0.01, or 0.001%) in 10% DMSO aq. (18) and vehicle controls were applied with a pipette and gently rubbed into the skin with cotton-tipped applicators, 1 for each preparation. The non-irritant concentrations selected for the ROATs were based on pre-tests carried out in FCA-treated control animals. Besides this, each experiment contained a sham-treated control group (IV and VIII respectively) where no allergen had been administered on induction.

Reading of treatment sites

When confluent erythema was obtained – defined as a “positive ROAT” (18) – the treatment was discontinued on that particular site. As in our previous studies, other reactions (papules, crusts, erosions, scaling) were also recorded but are not reported here. The test sites were read immediately before the next treatment, i.e., every 24th hour.

Table 2. Induction with PdCl₂; outcome of ROATs with 1% PdCl₂, NiSO₄ (0.1, 0.01 or 0.001%) and with 10% DMSO (vehicle); results in pre-ROAT patch testing with PdCl₂ and post-ROAT testing with PdCl₂ and NiSO₄

	Pre-ROAT patch testing; thresholds of sensitivity to PdCl ₂ at D24 (D3 reading)		ROAT: reactivity after 10 treatments (D45)		Post-ROAT (D60) patch test reactivity; no. pos animals (++, D3 reading)			
	conc. (%)	no. pos.	test preparation	no. pos.	PdCl ₂	NiSO ₄	pet.	simultaneous reactivity to PdCl ₂ and NiSO ₄
group I (n=9)	2.5	2	1% PdCl ₂	9	4	6	0	3
	1.25	2	0.1% NiSO ₄	2				
	0.625	3	10% DMSO	2				
	0.313	2						
	0.156	1						
group II (n=9)	2.5	2	1% PdCl ₂	9	6	2	0	1
	1.25	2	0.01% NiSO ₄	0				
	0.625	3	10% DMSO	2				
	0.313	2						
	0.156	1						
group III (n=9)	2.5	1	1% PdCl ₂	9	6	4	1	4
	1.25	3	0.001% NiSO ₄	0				
	0.625	2	10% DMSO	1				
	0.313	2						
	0.156	1						
group IV (controls) (n=11)	not tested		1% PdCl ₂	3	2	3	0	0
			0.1% NiSO ₄	0				
			10% DMSO	0				

Results

Induction with PdCl₂ – ROATs with PdCl₂ and NiSO₄ (Table 2)

All 27 animals were positive on pre-ROAT patch testing. After 10 treatments, 27/27 guinea pigs induced with PdCl₂ had reacted to 1.0% PdCl₂ (Fig. 1) and 2/9 to 0.1% NiSO₄ and to the vehicle – the same animals. None reacted to the lower concentrations of NiSO₄ (0.01 and 0.001%) but 3 animals reacted to the vehicle.

In post-ROAT patch testing, 16/27 (59%) guinea pigs reacted to PdCl₂ and 12/27 (44%) to NiSO₄. Among the 16 animals positive to PdCl₂, 8 were simultaneously positive to NiSO₄.

3 out of 11 control animals (27%) reacted late in the ROATs with PdCl₂ (Fig. 1). In post-ROAT patch testing, 2/11 of these reacted to PdCl₂ and 3/11 to NiSO₄ – not the same animals.

Induction with NiSO₄ – ROATs with NiSO₄ and PdCl₂ (Table 3)

All 30 animals were positive on pre-ROAT patch testing. After 10 treatments, 9/30 (30%) guinea pigs induced with NiSO₄ had reacted to 0.1% NiSO₄ (Fig. 1) and 7/30 (23%) to PdCl₂ (4 to 1% and 3 to 0.1% PdCl₂). In post-ROAT patch testing, 14/30 (47%) guinea pigs reacted to NiSO₄ and 5/30 (17%) to PdCl₂. 5 control animals (50%) reacted late in the ROATs with PdCl₂ (Fig. 1) but none to NiSO₄.

Reactivity in ROATs in relation to patch test results (Table 4)

Pre-ROAT. The results of patch testing (D24) were compared with reactivity in ROATs. The concordance (positive test and positive ROAT plus negative test and negative ROAT) was 100% for PdCl₂ and varied between 10 and 40% for NiSO₄.

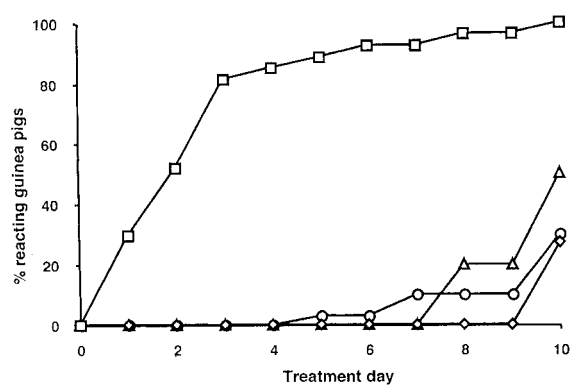


Fig. 1. Cumulative reactivity in ROATs (D35–44=treatment D0–9) in series with 1% PdCl₂ in saline (Table 2) and with 0.1% NiSO₄ in 10% DMSO (Table 3) induced according to the guinea pig maximization test method and demonstrating ++ and +++ reactions in pre-ROAT patch testing on D24. Sham-treated control animals reacted to PdCl₂ (group IV, Table 2 and group VIII, Table 3) but not to NiSO₄. —□— induction and ROAT: PdCl₂ (n=27); —△— control II, ROAT: PdCl₂ (n=10); —○— induction and ROAT: NiSO₄ (n=30); —◇— control I, ROAT: PdCl₂ (n=11).

Table 3. Induction with NiSO₄; outcome of ROATs with 0.1% NiSO₄, PdCl₂ (1, 0.1 or 0.01%) and with 10% DMSO (vehicle); results in pre-ROAT patch testing with NiSO₄ and post-ROAT testing with NiSO₄ and PdCl₂

	Pre-ROAT patch testing; thresholds of sensitivity to NiSO ₄ at D24 (D3 reading)		ROAT: reactivity after 10 treatments (D45)		Post-ROAT (D60) patch test reactivity; no. pos animals (++, D3 reading)			
	conc. (%)	no. pos.	test preparation	no. pos.	NiSO ₄	PdCl ₂	pet.	simultaneous reactivity to NiSO ₄ and PdCl ₂
group V (n=10)	1.0	5	0.1% NiSO ₄	1	4	2	0	0
	0.5	1	1% PdCl ₂	4				
	0.25	1	10% DMSO	0				
	0.125	1						
	0.067	2						
group VI (n=10)	1.0	4	0.1% NiSO ₄	5	3	1	1	1
	0.5	1	0.1% PdCl ₂	3				
	0.25	1	10% DMSO	0				
	0.125	1						
	0.067	3						
group VII (n=10)	1.0	4	0.1% NiSO ₄	3	7	2	0	1
	0.5	1	0.01% PdCl ₂	0				
	0.25	2	10% DMSO	0				
	0.125	2						
	0.067	1						
group VIII (controls) (n=10)	not tested		0.1% NiSO ₄	0	0	1	1	0
			1% PdCl ₂	5				
			10% DMSO	0				

Post-ROAT. The results of patch testing (D60) were compared with reactivity in ROATs. The concordance for PdCl₂ had decreased (range 56–78%) while it had increased for NiSO₄ (range 40–60%).

Discussion

The traditional way of studying cross-reactivity is patch testing (9) but, when the outcome of this method (12) is compared with the outcome of ROATs, divergent results were obtained, especially in guinea pigs induced with PdCl₂ (Table 5). The animals reacted to both PdCl₂ (100%) and NiSO₄ (80%) on patch testing (12), while only 2 reacted to NiSO₄ in the ROATs. It thus appears that the

outcome of patch testing overestimates the risk of a relapse.

For animals induced with NiSO₄, the results are harder to interpret, since only 30% of the patch-test-positive guinea pigs reacted in the ROATs (Fig. 1, Table 3), and with a rather low concordance (Table 4). Only 2 animals showed simultaneous reactivity to NiSO₄ and PdCl₂ on post-ROAT patch testing (Table 3). In previous cross-reactivity studies (12), it was found that in guinea pigs induced with NiSO₄ 87% reacted to NiSO₄ but none at all to PdCl₂ (Table 5).

In studies using cobalt chloride (CoCl₂), several animals did not react in ROATs despite a low sensitivity threshold (18). The concordance was concentration-dependent and varied between 47 and 88% in the concentration range 0.001 to 0.1% CoCl₂. It was suggested that reactivity in ROATs might be related to the barrier function at test sites (18), but in a study among patients sensitive to

Table 4. Concordance between outcome of ROATs and results of patch testing carried out pre-ROAT and post-ROAT

Group	ROATs with	Concentration (%)	% concordance ^{a)}	
			pre-ROAT	post-ROAT
I	PdCl ₂	1	100	56
II	PdCl ₂	1	100	78
III	PdCl ₂	1	100	67
IV	PdCl ₂	1	NT (controls)	73
V	NiSO ₄	0.1	10	60
VI	NiSO ₄	0.1	40	60
VII	NiSO ₄	0.1	30	40
VIII	NiSO ₄	0.1	NT (controls)	100

^{a)} Concordance: positive test and positive ROAT, plus negative test and negative ROAT.

NT: not tested.

Table 5. Comparison of patch test results for cross-reactivity (12) with the outcome of ROATs (Tables 2, 3)

Induced with	Patch testing			ROAT		
	no. tested	% reactivity		no. treated	% reactivity	
		PdCl ₂	NiSO ₄		PdCl ₂	NiSO ₄
PdCl ₂	10	100	80	27	100	7 ^{a)}
NiSO ₄	15	0	87	30	23 ^{a)}	30

^{a)} 3 concentrations of the allergen were studied.

colophony no impairment was found using measurements of transepidermal water loss (TEWL) (15). Thus other explanations must be sought as to why some guinea pigs and humans do not react in ROATs in spite of a low sensitivity threshold on patch testing.

The concentrations employed in the ROATs were based on pre-tests in sham-treated guinea pigs. Some control animals treated only with 1% PdCl₂ in ROATs reacted late (D8–10) (Fig. 1), indicating that the topical treatments had induced sensitivity. This assumption was confirmed in post-ROAT patch testing (Tables 2, 3). Thus, intradermal administration of PdCl₂ is not required, confirming our previous finding that it is a potent sensitizer in the guinea pig (16).

The reactivity to PdCl₂ on post-ROAT patch testing in animals induced with NiSO₄ (Table 4) can thus be ascribed to cross-reactivity as well as to the induction of palladium sensitivity by the repeated treatments.

On the other hand, ROATs with NiSO₄ were negative in the sham-treated control animals (group VIII, Table 3) and none reacted on post-ROAT patch testing. The reactivity to NiSO₄ observed on post-ROAT patch testing (groups I–III, Table 2) in animals induced with PdCl₂ thus indicates cross-reactivity and not the treatments.

An analogous study, where guinea pigs were induced with NiSO₄ or CoCl₂ and then treated topically (ROATs) with these allergens, will be reported separately (19). To evaluate our experimental findings a further step would be to carry out ROATs in man (13–15) with these 3 metal compounds.

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