

Iontophoresis of nickel elicits a delayed cutaneous response in sensitized individuals that is similar to an allergic patch test reaction

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Wearing of patch test chambers for 1–2 days is uncomfortable for patients. Allergen application by iontophoresis avoids this, but it is unknown so far whether iontophoresis itself interferes with the delayed immune response. We compared the effects occurring 48 h after iontophoresis with distilled water, 0.9% NaCl, and 0.01 M NiSO₄ in normal volunteers and in nickel-sensitized patients (total $n=36$). Visual assessment was performed and transepidermal water loss (TEWL), stratum corneum hydration, cutaneous blood flow, and immunohistopathology were determined. After iontophoresis with nickel sulfate, only individuals sensitized to nickel reacted with a positive clinical response, increase in cutaneous blood flow, decline in epidermal CD-1a-positive cells, increase in epidermal proliferation (Ki-67-positive cells), pronounced infiltration of cells positive for CD4, CD11, or CLA, and cellular activation (expression of ICAM1, HLA-DR). Iontophoresis with distilled water or saline did not result in such reactions in volunteers with or without nickel sensitization, and the latter also tolerated nickel iontophoresis without significant skin reactions. We conclude that the delayed cutaneous response to nickel induced via iontophoresis is specific and similar to a positive patch test reaction. Iontophoresis may therefore be considered as an alternative to patch testing.

Key words: diagnosis of contact allergy; immunohistopathology; transepidermal water loss; cutaneous blood flow; epidermal barrier. © Munksgaard, 2000.

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Patch testing is the time-honoured tool to diagnose contact hypersensitivity. It is safe, fairly well standardized and requires little technical equipment (1). However, the recommended attachment of patch test chambers for 2 days (1,2) is a nuisance for the patients; they have to avoid all activities that might interfere with the proper adhesion of the test chambers on their back during these days, and they are likely to experience some irritation (3). Therefore, a less uncomfortable method of allergen application is desirable.

Iontophoresis is a simple non-invasive technique by which many exogenous substances can rapidly be introduced into intact or functionally disturbed skin (4–11). Iontophoretic delivery of allergens, including nickel ions, is also possible (12–15) and had already been reported more than 50 years ago (16). More recently, cutaneous delayed reactions elicited by nickel iontophoresis were shown to cor-

relate well with positive patch tests in nickel-sensitized individuals (15).

However, iontophoresis itself (i.e., with allergen-free water) is known to change significantly certain skin functions like sweating and capillary perfusion (17–19), and electric current may induce histamine release from peripheral blood leucocytes (20), loss of the epidermal calcium gradient (21), or epidermal injury (22). Therefore, the cutaneous effects caused by iontophoretic delivery of an allergen need to be determined before iontophoresis can be considered as a substitute for patch test chambers.

For this purpose, we have compared the delayed effects caused by iontophoresis with purified water, saline and nickel in the skin of normal individuals and of persons allergic to nickel. The parameters determined were clinical reading, transepidermal water loss (TEWL), stratum corneum hydration, cutaneous blood flow, and immunohistopathology.

Patients and Methods

A total of 36 volunteers (20 women, 16 men; age 19–47 years) gave their informed consent to participate in this study, which was approved by the Ethical Committee of the Medical Faculty of the University of Kiel. 16 participants showed a positive patch test to nickel sulfate, the others were patch test negative and had no history of nickel allergy.

An iontophoresis chamber (ION 1, direct type) combined with an indifferent electrode MIC.WB1, a iontophoresis controller MIC 1 and a laser doppler-flowmeter MBF 3D (all from Moor Instruments LTD, Millwey, Axminster, Devon, UK) were used for the iontophoresis experiments. The iontophoresis chamber (cathode) was attached to the flexor side of one distal arm, located approx. 10 cm proximally to the indifferent wrist strap electrode (anode). The iontophoresis chamber was filled with the test fluid (distilled water, 0.9% NaCl in distilled water or 0.01 M NiSO₄ in distilled water). Owing to the construction of the chamber, a circular skin area of 0.71 cm² came into direct contact with the test fluid. The iontophoresis controller was set to allow a current flow of 33–37 μ A for 360 s between the electrodes in each experiment.

TEWL was measured with a Tewameter TM 210 (Courage and Khazaka, Germany) according to current guidelines (23), and stratum corneum hydration was determined by use of a Corneometer CM 820 PC (Courage and Khazaka, Germany).

Cutaneous blood flow was measured by use of a laser Doppler-flowmeter (MBF 3D, Moor Instruments LTD) and an optic probe P2 (Moor Instruments LTD) over a time period of 30 s under the same conditions as adhered to for TEWL measurements. According to the manufacturer's specification, the mean red blood cell flux (FLUX), red blood cell concentration (CONC) and mean blood cell speed (SPEED) were expressed in arbitrary units.

From the iontophoresis-treated skin areas

located directly under the iontophoresis chamber, 6-mm punch biopsies were taken under local anaesthesia. One half of each biopsy was fixed with formalin and subsequently embedded in paraffin for histopathology. The other half was shock-frozen in liquid nitrogen for subsequent cryostat sectioning and immunostaining with a panel of monoclonal antibodies (purchased from DAKO, Table 1) by use of a commercially available 3-step biotin-avidin immunoperoxidase kit (DAKO). Immunostaining was also performed with formalin-fixed biopsies after previous deparaffinization and unmasking of the antigenic epitopes if necessary.

For evaluation of infiltrating cells, microscopic slides with representative densities of inflammatory infiltrates were chosen from each biopsy. In the epidermis, the densities of cells positive for Ki 67 and CD 1a were determined by counting them within epidermal sections of defined length (0.55 mm and 0.35 mm resp.), using a 200-fold and 400-fold magnification and a calibrated ocular grid (Zeiss, Germany). The accumulation of cells positive for the other antibodies used in this study was assessed semi-quantitatively as negative (no cells), weak (1–5 cells), moderate (5–15 cells) or strong (>15 cells), separately for their aggregation in epidermal, papillary, perivascular, and dermal localizations. Negative, weak, moderate or strong staining with ICAM-1 was discriminated for papillary and dermal endothelial cells. Cytokeratin-staining was assessed as negative, weak or strong.

For statistical analyses (levels of TEWL, blood flow, and hydration, densities of Ki-67- and CD-1a-positive cells), Mann-Whitney *U*-statistics was used and two-tailed *p*-values were calculated. *p*<0.05 was considered statistically significant.

Results

Clinical response

Iontophoresis with the test solutions was well tolerated by all of the probands, although a mild stinging sensation was reported by some during iontophoresis. Particularly after iontophoresis

Table 1. List of primary monoclonal antibodies used

Antibody	Binding site	Target
DAKO M 721	CD 1a	LC, dendritic cells, cortical thymocytes
DAKO M 716	CD 4	T-helper cells, monocytes
DAKO M 732	CD 11c	macrophages, distinct circulating lymphocytes, hairy cells
DAKO M 701	LCA	lymphocytes, distinct macrophages and histiocytes
DAKO 7063	CD 54	ICAM-1-positive cells
DAKO M 746	HLA-DR	LC, activated T-cells, B-cells, macrophages
DAKO A 047	Ki 67	mitotic cells
DAKO M 3515	acidic and basic keratins	human cytokeratins (acidic: 56.5, 50, 50', 48, 40 kD; basic: 65–67, 64, 59, 58, 56, 52 kD)

Table 2. Transepidermal water loss in skin treated with iontophoresis (area in contact with test fluid) prior to and 48 h after iontophoresis with distilled water, 0.9% NaCl, and 0.01 M NiSO₄ in volunteers not allergic to nickel (controls) and in nickel-sensitive patients

	H ₂ O		NaCl		Nickel	
	0 h	48 h	0 h	48 h	0 h	48 h
Controls	17.6±3.6	18.5±3.3	17.0±2.9	18.5±3.3	17.4±4.8	18.2±3.3
Ni-sensitive patients	16.3±4.4	15.1±4.9	13.8±4.1	15.9±5.8	15.0±4.6	19.1±9.1

The data shown are arithmetic means ($n=7$) of TEWL in g/m²/h±standard deviation.

with distilled water, some probands developed an immediate erythema that disappeared within 30 min. Except for this early response, no further reactions were clinically observed in any probands after iontophoresis with water or saline or with nickel sulfate in volunteers not sensitized to nickel. In contrast, all nickel-sensitive patients developed a delayed reaction after nickel iontophoresis within the skin area that had been in contact with the test fluid. Within 48 h, erythema and palpable infiltration was noted, plus papules and microvesicles in some cases.

Transepidermal water loss

TEWL was measured in 7 individuals not sensitized to nickel and in 6 nickel-sensitive probands prior to iontophoresis and 48 h after iontophoresis (Table 2). Iontophoresis did not result in a significant alteration of TEWL in both groups when water or saline was applied. Iontophoresis with nickel sulfate resulted in a slight increase of TEWL in nickel-sensitive persons only (from a mean of 15.0±4.6 g/m²/h to 19.1±9.1 g/m²/h; Table 2), which did not reach significance ($p>0.1$).

Stratum corneum hydration was in the range between 45 to 53 units in all measurements and remained unchanged after 48 h in all probands following iontophoresis with water, saline and nickel sulfate, as compared to the corresponding hy-

dration measured prior to iontophoresis (data not shown in detail).

Cutaneous blood flow

Cutaneous blood flow was significantly increased 48 h after iontophoresis with nickel sulfate in nickel-sensitive patients only, as indicated by marked increases of FLUX (>10-fold), SPEED and CONC (>3-fold) ($p<0.001$). In contrast, no change in blood flow was seen in control probands and in nickel-sensitive patients after iontophoresis with water and saline (Table 3).

Immunohistopathology

Immunostaining of biopsies obtained from nickel-sensitive patients 48 h after nickel iontophoresis revealed marked differences as compared to biopsies taken from the control volunteers. The staining patterns of the latter were all very much alike.

In nickel-sensitive patients, the mean number ($n=9$) of intraepidermal CD-1a-positive cells per 0.35 mm epidermis was 3.5±1.5 after iontophoretic application of nickel sulfate, which is significantly lower ($p<0.05$) as compared to samples from patients not sensitive to nickel treated in the same way (5.4±1.1 cells/0.35 mm; $n=6$) or the cell density in nickel-sensitive patients treated with saline or water ($p<0.01$). The corresponding mean

Table 3. Cutaneous blood flow in skin treated with iontophoresis (area beneath iontophoresis chamber) prior to and 48 h after iontophoresis with distilled water, 0.9% NaCl, and 0.01 M NiSO₄ in volunteers not allergic to nickel (controls) and in nickel-sensitive patients

	H ₂ O		NaCl		Nickel	
	0 h	48 h	0 h	48 h	0 h	48 h
Controls						
FLUX	16.0±9.5	12.9±4.0	14.6±5.7	15.8±6.3	14.1±7.5	12.0±3.3
SPEED	13.4±6.6	10.7±3.2	15.3±8.2	14.0±3.2	11.2±3.9	11.8±3.3
CONC	62.5±14.2	63.1±14.7	58.6±24.3	59.3±19.6	70.0±35.1	57.7±18.0
Ni-sensitive patients						
FLUX	20.0±19.0	21.7±17.9	11.0±5.2	11.1±3.1	11.7±5.0	249.1*±135.0
SPEED	19.1±14.8	17.8±10.2	11.8±5.0	14.0±2.8	15.0±7.9	73.7*±26.2
CONC	50.5±15.4	57.9±22.1	50.2±14.1	40.3±6.6	45.0±7.0	165.6*±41.7

The data shown are arithmetic means (n between 7 and 9) of flow rate (FLUX), blood speed (SPEED) and concentration of erythrocytes (CONC) in arbitrary units±standard deviation.

*: $p<0.01$ for comparison with corresponding value at 0 h.

numbers ($n=4-6$) of CD-1a-positive cells per 0.35 mm epidermis in biopsies taken after iontophoresis with water or saline were 5.1 ± 0.4 (nickel-sensitive patients, dist.water), 5.8 ± 0.8 (nickel-sensitive patients, NaCl), 5.4 ± 1.0 (volunteers not sensitive to nickel, dist.water), and 5.4 ± 1.2 (volunteers not sensitive to nickel, NaCl).

Following nickel iontophoresis, the mean number of Ki-67-positive cells was significantly higher ($p < 0.01$) in biopsies from nickel-sensitive patients after iontophoresis with nickel sulfate than in biopsies from volunteers not sensitized to nickel (25.9 ± 14.6 , $n=7$, versus 9.6 ± 0.6 , $n=6$) or from nickel-sensitive patients treated with saline or water ($p < 0.01$). The corresponding mean numbers ($n=4-6$) of Ki-67-positive cells per 0.55 mm length of epidermis in biopsies taken after iontophoresis with water or saline were 10.9 ± 2.8 (nickel-sensitive patients, dist.water), 10.8 ± 2.3 (nickel-sensitive patients, NaCl), 9.7 ± 0.4 (proband not sensitive to nickel, dist.water), and 10.2 ± 0.9 (proband not sensitive to nickel, NaCl).

The average infiltration of CD-4-positive cells in biopsies from nickel-sensitive patients treated with nickel iontophoresis ($n=6$) was found to be moderate to strong within the epidermis, strong in the perivascular dermis, and weak to moderate in the interstitial and papillary dermis. In the biopsies of all other patients and volunteers ($n=4-6$) only a few CD-4-positive cells were seen near to blood vessels throughout, whereas CD-4-positive cells in other sites were rare.

Similarly, a moderate perivascular dermal accumulation of CD-11-positive cells was typical for all samples taken from nickel-sensitive patients treated with nickel iontophoresis ($n=6$); in this subgroup of patients a few CD-11-positive cells were usually also present in the epidermis and in the papillary dermis, whereas in all other patients and volunteers ($n=4-6$) occasional CD-11-positive cells were confined to the perivascular dermis.

Papillary vessels and deeper dermal vessels were markedly ICAM-1-positive (moderate to strong staining) in the biopsies from nickel-sensitive patients treated with nickel iontophoresis ($n=8$). In addition, there was a strong infiltration of ICAM-1-positive leucocytes in perivascular localization as well as in the papillary dermis of these biopsies, and in 3 of them ICAM-1-positive cells were also found within the epidermis. In the biopsies of all other patients and volunteers ICAM-1-positive blood vessels were also found preferentially in the papillary dermis (weak to moderate staining), but no ICAM-1-positive leucocytes were detected.

LCA-staining revealed a dense perivascular accumulation of positive cells and a weak to moder-

ate infiltration within the epidermis and papillary dermis in all biopsies taken from nickel-sensitive patients treated with nickel iontophoresis ($n=8$). In contrast, no positive cells were found in the biopsies obtained from all other probands.

In biopsies taken from nickel-sensitive patients after iontophoresis with nickel, expression of HLA-DR was found on intraepidermal dendritic cells and some other cells within the epidermis. In addition, there was a strong HLA-DR-positive perivascular and papillary cellular infiltrate. Blood vessels in particular were intensely stained with antibodies against HLA-DR. In comparison, there was strong positive staining of endothelia and of intraepidermal dendritic cells in all other biopsies, too, but a considerably smaller number of HLA-DR-positive cells were present in perivascular or papillary localizations.

Rather uniform staining, particularly of dermal eccrine sweat glands, was obtained with the antibodies against cytokeratins in all biopsies from all groups of patients, independent of nickel allergy and of the test fluid applied by iontophoresis.

Discussion

Iontophoresis has been used successfully for the transportation of allergens into the skin (12-15). In the present study we showed that delayed cutaneous responses after iontophoresis with nickel sulfate can be reliably induced in nickel-sensitive patients but will not occur in individuals without nickel allergy.

The main aim of our study was to compare iontophoretically elicited delayed cutaneous responses to an allergen (nickel ions), other ions (Na ions) and vehicle (distilled water), in order to see whether iontophoresis itself interferes with the delayed immune response. Although iontophoresis-induced alterations of cutaneous perfusion, epidermal calcium distribution, epidermal integrity and sweating have been observed (17-21), this question had not been addressed so far.

Our results show that the process of iontophoresis itself was not responsible for the effects observed in nickel-sensitized patients 48 h after treatment. Whereas these patients responded to nickel iontophoresis with a significant increase in their cutaneous blood flow and with considerable inflammatory cellular aggregation and activation, iontophoresis with distilled water or saline did not produce such reactions in patients with or without nickel allergy. Volunteers not sensitized to nickel also tolerated nickel iontophoresis without significant skin reactions. Iontophoresis itself did not produce profound delayed changes in barrier function, or of epidermal and dermal cellular aggre-

gation and activation as compared to normal skin (21, 24, 25).

Immediate reactions owing to iontophoresis itself, which occurred in some cases, were unrelated to nickel sensitization and to the test solution used for iontophoresis. Some of our patients developed an erythema that vanished within 30 min, and some reported a short stinging sensation. These effects may be due to initial nonspecific vasodilation and/or to histamine release caused by iontophoresis (18, 20).

We asked whether the delayed cutaneous reactions induced by nickel iontophoresis in nickel-sensitized patients were similar to positive patch test reactions to nickel. A statistically significant increase in TEWL was not seen in our nickel-sensitive patients treated with nickel-iontophoresis. In contrast, allergic patch test reactions are characterized by an increased TEWL (25–27), though the variability in this increase is considerable (28). The fact that in our study most of the positive reactions to nickel were weak reactions, according to ICDRG criteria (29), may explain our finding of a generally unchanged TEWL. The possibility that in iontophoretically elicited delayed-type allergic reactions the epidermal barrier could be less impaired than in patch test reactions is substantiated, however, by the unchanged epidermal hydration and by the unchanged staining of cytokeratins within the epidermis in our study.

The reduced number of epidermal Langerhans cells (CD-1a-positive cells) as well as the enhanced epidermal proliferation (Ki-67-positive cells) in delayed reactions to nickel correlate with previous findings in positive patch tests (25, 30–32) and the decrease in LC numbers is consistent with the supposed emigration of LC from the epidermis after antigen exposure (33).

Responses of the blood vessels were also determined in the iontophoretically elicited nickel reactions, as reflected by a significant increase in blood flow, strong expression of ICAM-1 and HLA-DR and a marked perivascular cellular infiltrate. These observations, as well as the presence of intraepidermal leucocytes and the results of our immunostaining, are similar to findings in allergic patch test reactions (24, 32, 34, 35–40).

Future studies should be performed with a broad spectrum of contact allergens besides nickel. Although iontophoresis works particularly well with small electrically charged particles like nickel ions, there is evidence that uncharged and larger molecules can also be transferred into the skin by iontophoresis (7, 11, 41). Another important question would be if the unsatisfactory reproducibility of conventional patch testing with some allergens (42–44) can be improved by iontophoresis.

In conclusion, our results indicate that iontophoretically elicited delayed cutaneous immune reactions to nickel are specific and similar to allergic patch test reactions. This indicates that iontophoresis itself does not interfere with the delayed immune response. The application of suitable test allergens by iontophoresis may therefore be a more comfortable alternative to the classical patch test procedure.

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