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Nickel Contact Sensitivity in the Guinea Pig

An Efficient Open Application Test Method

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Nickel contact sensitivity was successfully induced in guinea pigs using an open epicutaneous application method. Immediately after pretreatment with 1% aqueous sodium lauryl sulfate, upper back skin was treated daily for 4 weeks with 0.3%–3% nickel sulfate in either a 1% lanolin cream (Vaseline, pH 5 SAD crème) or hydroxypropyl cellulose. Weekly intradermal injections with aluminium potassium sulfate were used as adjuvant. The animals were challenged twice with a one week interval, with nickel sulfate 2% in water and 1% in petrolatum, respectively.

The response rates in the test groups treated with nickel sulfate 1% or 3% in the lanolin cream or 1% in hydroxypropyl cellulose were significantly different from the response rate in the control group. Considering both readings at both challenges, the frequency of sensitization was 57–93% (8 of 14 to 13 of 14 animals) in the group treated with 1% in the lanolin cream, 60–100% (9/15 to 15/15 animals) in the group treated with 3% in the lanolin cream, and 67–75% (8/12 to 9/12 animals) in the group treated with 1% in hydroxypropyl cellulose. Rechallenge of initially sensitized animals 10 weeks later confirmed that a lasting contact allergy had been obtained.

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More than 25 different animal methods have been applied to induce nickel contact sensitivity using epicutaneous, intradermal, or intramuscular administration of various nickel salts, with or without adjuvant stimulation (1). The most commonly used model is the Guinea Pig Maximization Test (GPMT) introduced by Magnusson & Kligman in 1969 (2). The results with this model have varied (3–8), and a reproducible, high frequency of sensitivity guinea pigs has not been obtained. Wahlberg (6) recommended the GPMT for nickel tests as a starting point because of the chance of obtaining at least some nickel-sensitive animals. He emphasized that it was difficult to recommend one animal method rather than another, as there were few comparative studies.

Epicutaneous nickel administration has been used with greater success. Lammintausta et al. (4–5) sensitized 11/22 and 4/7 of guinea pigs painted with 25% nickel sulfate 5 days a week for 4 weeks. Zissu et al. (7) sensitized 63–80% of the guinea pigs by combining daily epicutaneous application of $NISO_4$ 1% in a lanolin cream on sodium lauryl sulfate (SLS) pretreated skin with weekly intradermal injections of potassium alum used as adjuvant. SLS pretreatment has previously been used to enhance sensitization (9–11). The mechanism for the enhancing effect is not known, but may be due to an irritating effect on the skin combined with an improved bioavailability of the allergen.

In *in vitro* skin penetration studies with nickel through excised human skin, the use of various hydrogels as vehicle enhanced cutaneous bioavailability of nickel significantly, compared with the use of petrolatum. The highest bioavailability was obtained with a hydroxypropyl methylcellulose gel (12).

The purpose of the present investigation was to reproduce the guinea pig results obtained by Zissu et al. (7) using nickel sulfate 1% in a lanolin cream and to compare the results with tests using other concentrations (0.3–3%) and hydroxypropyl cellulose as vehicle.

MATERIAL AND METHODS

Animals

Eighty female outbred albino guinea pigs (Dunkin-Hartley, Møllegaard, Ll. Skensved, Denmark) weighing 350–450 g on receipt were housed in groups of 3 in plastic cages. The animals were kept on a 12-h photoperiod, at a room temperature of 21±1°C, a relative humidity of 55±5%, with food and water available ad libitum (standard guinea pig pellets, Altromin®, 3123, Chr. Petersen A/S, Ringsted, Denmark). As bedding, beech wood chips (Glamsbjerg Træindustri A/S, Glamsbjerg, Denmark) were used. The animals were randomly assigned to test and control groups, ear marked and allowed to adapt for one week before use. Hair was removed by clipping and shaving. All animals were weighed on day 0, 7, 14, 17, 21 and 26.

Chemicals

Nickel sulfate 1% and 3% ($NISO_4$, $6H_2O$, analytical grade (E. Merck, Darmstadt, Germany)) was prepared at the hospital pharmacy in a 1% lanolin cream (Vaseline, pH 5 SAD creme, The Counties' Medicine Registration Office, Landemærket 10, Copenhagen, Denmark). Nickel sulfate, 0.3%, 1% and 3%, was prepared in hydroxypropyl cellulose (HPC) at Kabi-Pharmacia (Hillerød, Denmark). Sodium Lauryl Sulfate (SLS) 1% in distilled water (Ph. Eur., 2nd edn.) and aluminium potassium sulfate dodecahydrate (analytic grade) (E. Merck, Darmstadt, Germany) 1% in sterile water were also prepared at the hospital pharmacy.

Procedure

The procedure described by Zissu et al. (7) was followed in detail.

Eighty animals divided into five test groups and one control group were treated as follows:

1. (n=15) control group (treated with both vehicles)
2. (n=14) 1% nickel sulfate in lanolin cream
3. (n=15) 3% nickel sulfate in lanolin cream
4. (n=12) 0.3% nickel sulfate in HPC
5. (n=12) 1% nickel sulfate in HPC
6. (n=12) 3% nickel sulfate in HPC

Aliquots of nickel sulfate preparation (0.5 ml) were administered epicutaneously to a clipped, shaved and SLS pretreated skin area of 2×4 cm located on the upper back of the animals (Fig. 1). The SLS

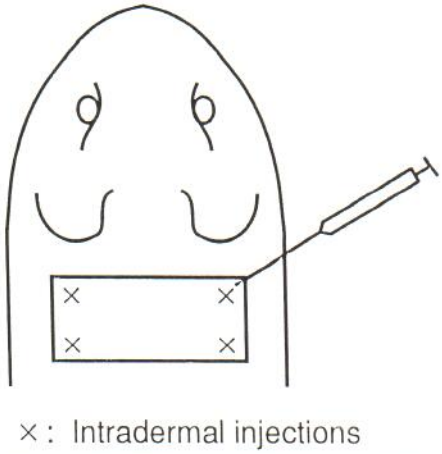

Fig. 1. The area for the daily topical nickel application and the weekly adjuvant (aluminium potassium sulfate) injections.

Table I. *Frequency of positive animals in each group after challenge with nickel sulfate 2% in water and 1% in petrolatum, respectively (number of animals with ++ or +++)*

	Challenge Preparation					
	2% $NISO_4$ in water			1% $NISO_4$ in petrolatum		
	Day 2	Day 3	Both	Day 2	Day 3	Both
Induction treatment:						
1% lanolin cream	12/14**	13/14***	12/14***	8/14**	9/14*	8/14**
3% lanolin cream	9/15	15/15***	9/15*	11/14***	10/14**	9/14**
0.3% HPC	3/12	6/12	3/12	5/12	8/12*	5/12
1% HPC	9/12*	9/12*	9/12**	8/12**	8/12*	7/12*
3% HPC	6/12	8/12*	6/12	3/12	3/12	1/12
Control	3/15	3/15	2/15	1/15	2/15	1/15

Significantly different from the control group (Fisher's exact test): $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$.

pretreatment was performed immediately before the treatment with nickel sulfate. They were treated five times a week for 4 weeks (Tuesday, Wednesday, Thursday, Friday and Sunday). On the first day of each week the animals received four intradermal injections (each 0.125 ml) with aluminium potassium sulfate as adjuvant (Fig. 1). These injections caused no skin necrosis or visible inflammation.

The skin painting resulted in skin irritation in some animals which required interruption of the treatment until recovery for various periods of time, as indicated in Table II. One animal in group No. 3 never recovered completely and was sacrificed between the two challenges.

The animals were challenged 14 and 21 days after the last treatment (days 42 and 49 from the first day of induction) by occlusive patch tests on the flank, using Finn Chambers® (Epitest Ltd., Helsinki, Finland) on Scanpor® (Norgesplaster A/S, Norway) fixed with Acrylastic® and Leucoflex® (both from Beiersdorf AG, Germany). The challenge concentration was nickel sulfate 2% in distilled water on day 42 as performed by Zissu et al. (7), and 1% in petrolatum on day 49 as used in our first experiment (8). The challenge concentrations were non-irritant after performance of a pilot study on naive guinea pigs.

The challenge reactions were read 'blind' after 2 and 3 days. The following grading scale was used: 0: no visible reaction, +: discrete or patchy erythema, ++: moderate or confluent erythema, +++: erythema and swelling (2). A grade + reaction was not regarded as a positive reaction. The number of sensitized animals (grades ++ and +++) in each group were used in the statistical analyses.

After the last challenge, 30 animals were selected (9 controls and 21 highly sensitive animals) for continued study and a rechallenge 10 weeks later to examine the duration of the sensitization.

Table II. *The frequency of nickel sensitive animals at both challenges and the number of treatments which were omitted in each group during induction due to skin irritation*

	Positive at both challenges	Treatments omitted/total number of treatments (%)
1% lanolin cream	9/14***	29/280 (10.4)
3% lanolin cream	12/14***	61/300 (20.3)
0.3% HPC	5/12*	1/240 (0.4)
1% HPC	7/12**	14/240 (5.8)
3% HPC	4/12	7/240 (2.9)
Control	0/15	0/300 (0)

Significantly different from the control group (Fisher's exact test): $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$.

Statistics

The results were reduced to either a positive or negative response and statistics for contingency tables were used. Due to small numbers Fisher's exact test was chosen instead of the χ^2-test.

RESULTS

Table I shows the frequencies of positive animals read twice in each group after the challenge with 2% nickel sulfate in water or 1% in petrolatum. A positive response was read in 6.7–20% of the control animals (1–3 out of 15 animals) indicating some skin irritation, the 2% in water being the worse.

The test groups treated with either nickel sulfate 1% or 3% in the lanolin cream or with 1% hydroxypropyl cellulose show response rates which are significantly higher than in the control group. A positive response at one reading was read in 57–93% (8–13 of 14) of the animals treated with 1% in lanolin cream, in 60–100% (9–15 of 15) of the animals treated with 3% in the lanolin cream, and 67–75% (8–9 of 12) of the animals treated with 1% in hydroxypropyl cellulose. Although the highest significance levels were obtained in the 1% lanolin cream group, there was no statistically significant difference when the group treated with nickel sulfate 1% in lanolin cream was compared with the group treated with 1% in hydroxypropyl cellulose. The response rates in the groups treated with nickel sulfate, either 0.3% or 3%, in hydroxypropyl cellulose were lower, as a significant difference from the control group was obtained only for the 0.3% group in the day 3 reading after testing with 1% in petrolatum, and for the 3% group in the day 3 reading after testing with 2% in water. When the 3% lanolin cream group was compared with the 3% hydroxypropyl cellulose group, the response rate of the lanolin cream group was higher at all four readings, but there was only a significant difference between the two groups in the day 3 reading after challenge with 1% in petrolatum. Finally, Table I shows that most of the positive responses found in the day 2 reading were present in the day 3 reading.

Table II shows in the first column the frequency of animals

positive at both challenges (days 42 and 49). None of the control animals was positive at both challenges. When the lanolin cream was used as vehicle, 64% (9/14) were positive in the 1% group and 85% (12/14) in the 3% group. When hydroxypropyl cellulose was used, 41% (5/12) were positive in the 0.3% group, 58% (7/12) in the 1% group and 33% (4/12) in the 3% group.

The second column shows the total number of times the treatment had to be omitted in each group due to skin irritation. No irritation was seen in the control group; most was seen in the 3% lanolin cream group. Nickel sulfate 3% in hydroxypropyl cellulose was significantly less irritating than nickel sulfate 3% in the lanolin cream ($p < 0.001$; χ^2-test).

The total weight increase during the 4 weeks of induction treatment was 42.6±5.1% for the control group, 32.3±7.3% for the 1% lanolin cream group, 23.7±8.6% for the 3% lanolin cream group, and 35.5±7.2%, 31.6±10.5%, 31.9±7.2% for the 0.3%, 1% and 3% HPC groups, respectively. The weight gain in all test groups was significantly less than in the control group (one-tailed Wilcoxon-Mann-Whitney test; $p < 0.002$).

Ten weeks after the last challenge, 9 control animals and 21 positive test animals were rechallenged with 1% in petrolatum. All test animals were positive (days 2 and 3) as well as one of the control animals, indicating a long-lasting sensitivity.

DISCUSSION

Open topical treatment with nickel sulfate 1% in either a lanolin cream or hydroxypropyl cellulose is suitable for the induction of nickel allergy in guinea and the contact allergy seems to be long lasting. As hydroxypropyl cellulose failed to induce sensitization at the 3% level, we recommend the lanolin cream as vehicle, thus the results reported by Zissu et al. (7) were reproducible.

Repeated open application of nickel sulfate at the concentrations 1% and 3% in both vehicles were close to or above the irritation limit when administered on SLS-pretreated skin in combination with weekly adjuvant injections with potassium alum.

The lower weight gain of the animals in the test groups suggests that nickel toxicity affects the animals and that the degree is dose related. The skin irritation observed in some of the control animals when challenged could possibly be due the angry skin phenomenon caused by potassium alum or the SLS treatment. The challenge concentrations should be defined in animals pretreated with SLS and aluminium potassium sulfate.

Although the design was not suited to perform dose-response relationships, we found that the 3% HPC group responded less well than the 1% HPC group. This is compatible with the results from a study of the dose-response relationship for the induction of formaldehyde contact sensitivity in the GPMT (13). The dose-response curve was non-linear, indicating that there was an optimal induction dose above and below which a decreased response rate was seen. This so-called 'overload' phenomenon seems to be a more generalized phenomenon (14). The level of contact sensitivity in guinea pigs is suggested to be determined by a balance between activated effector and suppressor cells, and the balance is influenced by the dose given (15). When using the lanolin cream as vehicle, a similar drop in sensitivity with increasing dose was not observed. The response in the 3% lanolin cream group was at the same level as the response in the 1% lanolin cream group, even though about 20% of the treatments in the 3% group had to be omitted due to skin irritation. This discrepancy between the results with the two vehicles may be attributable to a difference in bioavailability of the allergen or by the different irritation potential of the preparations. The penetration rate of nickel through human skin *in vitro* was higher when the nickel was applied in hydroxypropyl methylcellulose than in petrolatum (12). The bioavailability of nickel from the hydroxypropyl cellulose (HPC) used in our study may be similar to that from hydroxypropyl methylcellulose, and the bioavailability of nickel from the lanolin cream may be similar to that from petrolatum, suggest a higher biovailability of nickel from the HPC than from the lanolin cream. However, there seems to be no simple relationship between sensitization rates and the bioavailability of an allergen (16).

The sensitivity obtained has been shown to last for at least 10 weeks, as all the nickel-sensitive animals retested were positive. The single animal from the control group became positive either due to irritation or to sensitization following the two previous challenges.

The present results showing the possibility of inducing persistent nickel sensitivity in guinea pigs give us the go-ahead to study nickel bio-kinetics in allergic/non-allergic animals. Suction blister fluid from nickel-allergic women contained significantly less nickel than the suction fluid from matched controls without any known allergy (17). This suggests that cellular uptake of nickel in sensitized patients may affect the nickel bio-kinetics and distribution in the body. Animal experiments to study this question are pending.

Compared with other animal sensitization methods with regard to cost/benefit and staff intensiveness it should be mentioned that although the animals were treated for 20 days during 4 weeks, the amount of time spent each treatment day was relatively short because the materials were easy to apply and no bandaging and only few injections were necessary.

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REFERENCES

1. Wahlberg JE. Nickel: Animal sensitization assays. In: Maibach HI, Menné T, eds. Nickel and the Skin: Immunology and Toxicology. Florida: CRS Press, Inc., 1988; 65–73.
2. Magnusson, B, Kligman AM. Allergic contact dermatitis in the guinea pig. Springfield, Ill., Charles C. Thomas, 1970.
3. Goodwin BFJ, Crevel RWR, Johnson AW. A comparison of three guinea-pig sensitization procedures for the detection of 19

reported human contact sensitizers. Contact Dermatitis 1981; 7: 248–158.
4. Lammintausta K, Kalimo K, Jansén CT. Experimental nickel sensitization in the guinea pig: comparison of different protocols. Contact Dermatitis 1985; 12: 258–262.
5. Lammintausta K, Korhonen K, Jansén CT. Method of sensitization determines if UVB irradiation inhibits the development of delayed type hypersensitivity to nickel in guinea pigs. Photodermatol 1986; 3: 102–103.
6. Wahlberg JE. Sensitization and testing of guinea pigs with nickel sulfate. Dermatologica 1976; 152: 321–330.
7. Zissu D, Cavelier C, de Ceaurriz J. Experimental sensitization of guinea pigs to nickel and patch testing with metal samples. Food Chem Toxicol 1987; 25: 83–85.
8. Rohold AE, Nielsen GD, Andersen KE. Nickel sulfate induced contact dermatitis in the guinea pig maximization test: a dose response study. Contact Dermatitis 1991; 24: 35–39.
9. Maurer T, Thomann P, Weirich EG, Hess R. Predictive evaluation in animals of the contact allergenic potential of medically important substances. II. Comparison of different methods of cutaneous sensitization with "weak" allergens. Contact Dermatitis 1979; 5: 1–10.
10. Möller H. Attempts to induce allergy to nickel in the mouse. Contact Dermatitis 1984; 10: 65–68.
11. Kligman AM. The SLS provocative patch test in allergic contact sensitization. J Invest Dermatol 1966; 46: 573–583.
12. Fullerton A, Andersen JR, Hoelgaard A. Permeation of nickel through human skin in vitro – effect of vehicles. Br J Dermatol 1988; 118: 509–516.
13. Andersen KE, Boman A, Vølund A, Wahlberg JE. Induction of formaldehyde contact sensitivity: Dose response relationship in the guinea pig maximization test. Acta Derm Venereol (Stockh) 1985; 65: 472–478.
14. Roberts DW, Basketter DA. A quantitative structure activity/dose response relationship for contact allergic potential of alkyl group transfer agents. Contact Dermatitis 1990; 23: 331–335.
15. Marcher E, Chase MW. Studies on the sensitization of animals with simple chemical compounds. XII. The influence of excision of allergenic depots on onset of delayed hypersensitivity and tolerance. J Exp Med 1969; 129: 103–121.
16. Andersen KE, Carlsen L, Egsgaard H, Larsen E. Contact sensitivity and bioavailability of chlorocresol. Contact Dermatitis 1985; 13: 246–251.
17. Bonde I, Beck H-I, Jørgensen PJ, Grandjean P, Brandrup F. Nickel in intercellular fluid. Acta Derm Venereol 1990; 70: 300–303.