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Zinc effects on nickel dermatitis in the guinea pig*

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Prevention of $NiSO_4$ induced allergic contact dermatitis (ACD) using $ZnSO_4$ in drinking water was studied in a guinea pig model. Without $ZnSO_4$ intervention, nickel (Ni)-exposure resulted in significantly higher ($p < 0.05$) stimulation indices (SIs) as compared to non-exposed controls, using $NiSO_4$ as an allergen in the lymphocyte transformation test (LTT). Oral intake of $ZnSO_4$ at both 250 μg/ml double-distilled deionized water (DDD) and 500 μg/ml DDD resulted in lower SIs than those of control guinea pigs drinking only DDD; the 250 μg $ZnSO_4$/ml group had significantly lower SIs ($p = 0.025$) than controls. There was no significant correlation between intradermal test responses and the SI values of individual guinea pigs exposed to $NiSO_4$. Mean zinc (Zn) concentrations in skin and in whole blood were not statistically different between the $NiSO_4$ exposed control and Zn supplemented groups, nor between Ni-sensitive and non-sensitive animals within groups. The rôle of Zn homeostasis, rôle of the Langerhans cell, effect of Zn supplementation on Ni ACD in other species, and possible blocking effects of other metals should be investigated in future studies.

Key words: allergy; dermatitis; nickel; zinc; lymphocyte transformation test; guinea pig maximization test; blocking effect; dietary supplementation.

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Nickel is a leading cause of allergic contact dermatitis (ACD) in many industrial nations (1–3). Epidemiologic studies of human patch testing with nickel sulfate ($NiSO_4$) revealed a prevalence of Ni allergy from 5–6% in the general public (4, 5) to 11% in dermatology clinic patients (6).

Spruit et al. (7) reviewed problems associated with various approaches to treating and preventing Ni ACD, but attempts to treat or prevent Ni ACD by exploiting metal:metal interaction were not found in the literature. Because of a possible blocking effect of another metal and because an excess or deficiency of an essential metal might contribute to increased resistance or susceptibility to Ni ACD, it was decided to explore such possibilities in the guinea pig ACD model.

Guinea pigs have successfully been sensitized to $NiSO_4$ using different protocols (8–11), the most sensitive of which is the guinea pig maximization test (GPMT) of Magnusson & Kligman (10). This test shows a high degree of correlation with similar tests in humans (12–13) and is an accepted procedure for identifying weak allergens, including $NiSO_4$ (14).

Nickel and other metals that cause ACD penetrate the skin and act as haptens, complexing with selected peptide/amino acid ligands to distort intercellular or cellular proteins, stimulating a Type IV delayed (cell-mediated) hypersensitivity (15–16). A chemically similar

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metal such as zinc should theoretically be able to block the site(s) where Ni complexes to proteins (17–18).

Zinc was chosen for this tudy for several reasons: Zn is in the 4th period of elements which includes Ni; Zn has been shown to interact with Ni in vitro and in vivo (19,20); and Zn interaction has been noted during animal feeding trials, with various purified diets and selected mineral supplements (21–22). Hill (23) found that oral Ni toxicity in chicks could be prevented by increased dietary Zn. Zinc has also been shown to interact with cadmium (24, 25) and other heavy metals (26).

Zinc and, to a lesser degree, Ni are both concentrated (20–38% of total Zn) in animal and human skin and ectodermal derivatives (27, 28). Under certain physiological conditions, sweat may constitute a significant excretory route for both Ni and Zn (29, 30). Zinc is recognized as a keratogenic element and is considered by many clinicians and authors to be essential for optimal wound healing (31). Various forms of Zn therapy are beneficial in treating a wide range of dermatological problems (27, 29).

Therefore, this study was undertaken to: (i) determine if Zn supplementation in drinking water during Ni sensitization and challenge had an effect on Ni-induced ACD in the guinea pig, (ii) compare in vitro lymphocyte transformation test responses with in vivo intradermal hypersensitivity test responses in Ni exposed and nonexposed guinea pigs, and (iii) determine if Zn supplementation resulted in increased concentrations in skin and whole blood that could be correlated with the amount of Zn supplement and the guinea pigs' Ni sensitivity status.

Material and Methods

Experimental animals

Non-pregnant female Hartley stock guinea pigs, 200–300 g, were housed individually in transparent, solid-bottomed, polycarbonate cages containing heat-treated, screened soft-wood bedding. The cages were fitted with stainless steel tops which accomodated a stainless steel J-feeder and 2 water bottles.

Guinea pigs were given commercial guinea pig pellets and tap water ad libitum for a 7–10 day acclimation period. Four lots of pelleted feed were used during the experiment and contained mean μg/gm ($\pm$SE) amounts of the following essential elements: Ca 10,075$\pm$363; P 7,700$\pm$378; Cu 19.2$\pm$1.1, Mn 131.3$\pm$8.1, Zn 126.8$\pm$8.2; Mo 2.4$\pm$0.1, and Ni 4.3$\pm$0.3. The mean ($\pm$SE) concentrations (μg/ml) of essential elements in two samples of tap water, one collected at the beginning and one at the end of the experiment, were: Ca 29.5$\pm$3.5; Cu $<$0.002; Mn 0.002$\pm$0.001, Zn 0.35$\pm$0.05; and Ni $<$0.001.

Double-distilled, deionized water (DDD) of 18-MΩ-cm resistance was used throughout the experiment for preparing drinking water supplements, reagents, media, and for rinsing glass and plastic ware used in handling samples for flame atomic absorption (AA) spectrophotometry. Two samples of this water, one collected at the beginning and one collected at the end of the experiment, were analyzed by the Ohio Department of Health, Water Chemistry Laboratory, and contained the following mean ($\pm$SE) concentrations (μg/ml) of these essential metals: Ca $<$5.0; Cu 0.55$\pm$0.05; Mn $<$0.03; Zn $<$0.03; and Ni $<$0.10.

Zinc supplementation in drinking water

Following the acclimation period, guinea pigs were randomly assigned to one of 4 groups: [I] 250 μg $ZnSO_4$/ml DDD, [II] 500 μg $ZnSO_4$/ml DDD, [III] DDD (Ni-exposed controls), or [IV] tap water (non-exposed controls). Zinc sulfate heptahydrate ($ZnSO_4$ $7H_2O$) was used (Mallinckrodt, Inc., St. Louis, MO). Zinc supplemented DDD, DDD alone, and tap water were the sole sources of drinking water for each group from 4 weeks before sensitization through the challenge phase of the experiment.

Nickel sensitization of guinea pigs

Guinea pigs received their assigned water for

4 wk and then were exposed (sensitization) to Ni ($NiSO_4$ $6H_2O$); Mallinckrodt, Inc., St. Louis, MO) by the intradermal and topical routes. The GPMT was modified by substituting non-ionic Triton X-100 (alkyl phenoxy polyethoxy ethanol) for sodium lauryl sulfate, as the topical surfactant or wetting agent (11). The Ni sensitization period was from day 0 through day 22. On day 23, the animals were challanged with $NiSO_4$ intradermally. Lymphocytes were collected and the guinea pigs were sacrificed on day 25.

The first exposure to Ni was by intradermal injection in the scapular area (day 0). Injections of 0.1 ml were made with Freund's complete adjuvant (FCA) emulsified with an equal volume of physiologic saline; 1% $NiSO_4$ $6H_2O$ (w/v) in physiologic saline; and 2% $NiSO_4$ $6H_2O$ (w/v) in physiologic saline emulsified with an equal volume of FCA (1% $NiSO_4$ in FCA). A 4×6 cm area on the dorsal midline over the scapular area was clipped of hair. Three pairs of intradermal injections were made 2 cm lateral and parallel to the midline (one row of three injections on each side of the midline) to sensitize test animals. Injections were made within the clipped area cranial-to-caudal in the following order: (i) 0.1 ml of FCA emulsion; (ii) 0.1 ml of 1% $NiSO_4$ $6H_2O$, and (iii) 0.1 ml of 1% $NiSO_4$ $6H_2O$ in FCA. Because of the high viscosity of FCA emulsions, a 1-ml glass tuberculin syringe fitted with a disposable 26 g, 3/8″ tuberculin needle (Becton Dickinson and Co., Rutherford, NJ) was used. Guinea pigs in the non-exposed control group (tap water) received the following injections: (i) 0.1 ml FCA emulsion; (ii) 0.1 ml physiologic saline; and (iii) 0.1 ml FCA emulsion.

On day 6, the scapular area was again clipped and then shaved with an electric shaver. A mixture of 1% Triton X-100 in white petrolatum, USP (w/w) was massaged with wooden applicator into the 4×6 cm area of bare skin to provoke a mild inflammatory reaction which favors sensitization (9). Triton X-100 was used in this series of experiments, as the surface active agent, because it is non-ionic and has been used successfully to sensitize guinea pigs to $NiSO_4$ (11).

On day 7, 5% $NiSO_4$ $6H_2O$ in petrolatum (w/w) was spread in a thick even layer onto a 3×5 cm piece of non-adherent surgical dressing. The $NiSO_4$-petrolatum patch was placed over the shaved skin and held by adhesive tape. This was secured by an elastic adhesive bandage twice the size of the patch. Non-exposed controls (tap water) was treated in the same manner, except Ni was not added to the petrolatum. The occlusive topical patch was removed after 48 h, on day 9

Intradermal challenge

On day 23, the right flank of each guinea pig was clipped and shaved. One-tenth ml intradermal injections of 0.25, 0.125, 0.0625, and 0.03125% $NiSO_4$ $6H_2O$ in physiologic saline (w/v) and 0.9% NaCl (control) were placed in a row into the flank. The diameter of distinct, well-circumscribed erythematous reactions was measured in millimeters (mm) with a transparent plastic grid 48 h after injection (11). Induration was not always pronounced and was not recorded. All sites were measured in the same room, under the same artificial lighting, by the same technician who was intentionally not told which were treatment or control groups.

Collection of blood and separation of peripheral blood lymphocytes

On day 25, blood was collected by percutaneous cardiac puncture with a disposable 10 ml plastic syringe containing 200 units of heparin. 8 ml of heparinized blood was placed into 24 ml (1 4 dilution) of calcium- and magnesium-free Hank's balanced salt solution (CMF-HBSS) containing 5 mM disodium dihydrogen ethylene diaminetetra-acetate dihydrate (EDTA) (32). Up to 4 ml of heparinized blood was retained for a complete blood count and/or frozen at $-18°C$ for later Zn determinations.

The lymphocytes were separated using a

combination of 10.00 vol Hypaque sodium 50%® (Winthrop Laboratories; New York, NY) added to 27.86 vol Ficoll-based Lymphoprep® (Nyegaard and Co. A/S, Oslo, Norway). The resulting solution had a density of 1.085 g/ml. Following centrifugation at $400 \times g$ for 40 min at room temperature, cells in the translucent layer at the plasma-gradient interface were removed with a sterile Pasteur pipet. These cells were washed in 22.5 ml heparinized (2 units/ml) CMF-HBSS and centrifuged in 30-ml round-bottomed polycarbonate (Oak Ridge-type) tubes at $200 \times g$ for 10 min. Pelleted cells were resuspended in 25 ml CMF-HBSS and centrifuged at $200 \times g$ for 10 min. After this second wash, pelleted cells were resuspended in 1.8 ml of RPMI 1640 medium with L-glutamine supplemented with 10% membrane-filtered, cell culture grade, heat-inactivated fetal bovine serum and 50 μg/ml gentamicin sulfate. Nucleated cells from each concentrate were counted in a hemacytometer chamber. Cell viability, by the trypan blue dye-exclusion test (33) ranged between 94.0–99.0%. Lymphocyte concentrates from each guinea pig were then adjusted to 1×10^6 live cells/ml with the RPMI 1640 supplemented medium.

Lymphocyte transformation test

Lymphocyte concentrates standardized to 1×10^6 live cells/ml were cultured in sterile, 96-well, flat-bottomed microtiter plates for 5 days at 37°C in a humidified atmosphere of 5% CO_2 in air. The CO_2 content of the incubation chamber was monitored using a Fyrite® atmospheric CO_2 analyser (Bacharach Instrument Co; Pittsburgh, PA). Each of 5 wells of the microtiter plate was seeded with 1×10^5 live lymphocytes (100 μl of standardized suspension); 0.5 μg mucophyto-hemagglutinin (PHA-M) from Difco Laboratories, in 100 μl medium, was a positive mitogenic control well; and $NiSO_4$ $6H_2O$ was added in 100 μl medium to give final concentrations of 5.40×10^{-6}, 2.70×10^{-6}, 1.08×10^{-6} M in 3 allergen wells. 2 higher concentrations (2.70×10^{-5} and 1.08×10^{-5} M $NiSO_4$ $6H_2O$) were used in early tests but they were discontinued because these concentrations appeared to be cytotoxic. A negative control well had 100 μl medium added. All LTT wells were run in quadruplicate for each guinea pig. 18 h before the end of the 5-day incubation period, 0.5 μCi tritiated-methyl-thymidine (^{3}HTdR) in 50 μl medium was added to each well. At the end of the 5-day incubation period, cells were harvested onto glass microfiber filter strips, using a semi-automatic multiple-well cell harvesting unit and dried overnight at room temperature in a drying oven. Resulting circular filter discs were placed in 20-ml glass scintillation vials containing 10 ml of a toluene-based scintillation cocktail and counted in a liquid scintillation system for 10 min.

Stimulation indices (SIs) were calculated for each concentration of allergen for each animal, using the following formula; each value being the arithmetic mean of quadruplicate determinations in counts per minute (cpm):

$$SI = \frac{\text{allergen well cpm} - \text{background well cpm}}{\text{control well cpm} - \text{background well cpm}}$$

Background cpm originated from "blank" microfiber filter discs in 10 ml of the cocktail. Stimulation indices of 2.0 or greater were considered indicative of sensitivity to $NiSO_4$ because $NiSO_4$ is considered a weak or moderately weak sensitizer of guinea pigs (11) and humans (34).

Analyses of tissue samples for zinc

On day 25, 2 to 4 ml of heparinized blood from each guinea pig was placed into a snap-top polystyrene test tube and frozen at −18°C. Following sacrifice, the left rear quadrant of each animal was closely clipped of all hair. A rectangle of skin, approximately 5×6 cm, was removed from over the loin and dorso-caudal hip, stored in a sealed polyethylene sample bag and frozen at −18°C.

Randomly selected blood and skin samples from both Ni-sensitive and nonsensitive guinea

pigs were thawed at room temperature. 2 ml of whole blood and 4 gm (maximum) of skin were placed in 17 or 30 ml porcelain crucibles, respectively, and covered with a matching 44 mm porcelain lid. Crucibles were then placed in a convection-type drying oven and heated at 65°, 95°, 145°, 195°, and 225°C for approximately 8 h at each setting, in succession. The samples were dry-ashed overnight at 550°C in a muffle furnace; then 300 μl of 30% hydrogen peroxide followed by 200 μl of 70% analytical grade HNO_3 was added to each crucible. An additional 9.5 ml of 1 N analytical grade HNO_3 was added to the crucibles and the resulting solution was decanted into 1-oz polyethylene bottles. Blood samples were further diluted to 15 ml, and skin samples to 25 ml, with 1 N HNO_3. Dilution of samples was based on expected levels of Zn in blood and skin (35–37). Flame AA analyses of guinea pig skin and blood samples were performed with a Varian, Model AA-775, double-beam atomic absorption spectrophotometer equipped with a deuterium lamp for simultaneous background correction of nonatomic absorption.

The least-squares equation resulting from calibration of spectrophotometer with Zn standards, for each batch of samples, was used to calculate the Zn concentration in diluted samples. These values were corrected for dilution factors, and Zn was expressed as μg/g (wet weight of skin) or μg/ml (blood).

Statistical methods

The two-tailed independent-samples *t*-test (38) was used to test a null hypothesis of no difference in means of guinea pigs between groups, or of Ni-sensitive versus non-sensitive animals within a treatment or control group. Stimulation indices were calculated for each of the 3 $NiSO_4$ concentrations in the lymphocyte transformation test. The Wilcoxon rank sum one-tailed test (38) was employed using the highest SI value for each guinea pig from two independent groups to test a null hypothesis that underlying population distributions were identical. Tests of the null hypothesis that a population linear correlation (38) equals zero were also conducted for in vitro and in vivo data. All tests were evaluated for significance at the 0.05 level.

Results

Lymphocyte transformation test

Allergen concentrations of 2.70×10^{-5} and 1.08×10^{-5} M $NiSO_4\ 6H_2O$ suppressed transformation/blastogenesis (Fig. 1). At these high concentrations, the SIs were < 1.76, while the 3 lower concentrations (5.40×10^{-6}, 2.70×10^{-6}, and 1.08×10^{-6} M) produced SIs equal to 4.80. The 2 highest $NiSO_4$ concentrations produced significantly lower SIs when compared to the other concentrations by the one-tailed Wilcoxon rank sum test ($p < 0.025$), and they were excluded in subsequent tests.

Of 37 guinea pigs exposed to Ni by the GPMT, 9 (24.3%) had at least one of three SIs ≥ 2.0. None of the non-exposed control (tap water) animals had an SI > 1.67 Using the highest SI for each guinea pig, and ranking them in the Wilcoxon test, the group consuming DDD (Ni-exposed controls) had significantly higher values ($0.025 < p < 0.05$) than the non-exposed control group.

The effects of $ZnSO_4$ consumption in drinking water, during intradermal and patch Ni

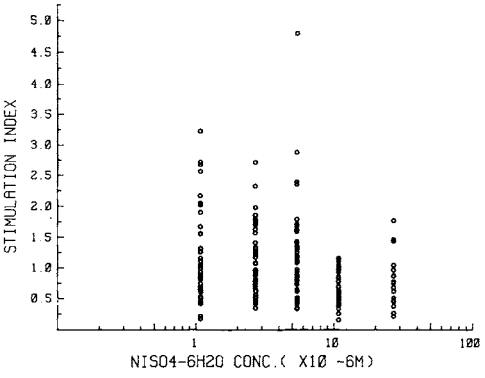

Fig. 1. Peripheral lymphocyte stimulation index for nickel-exposed guinea pigs, by concentration of $NiSO_4 \cdot 6H_2O$ used in lymphocyte transformation test.

Table 1. Mean counts per min (cpm)[a] in transformation tests of female guinea pig[b] lymphocytes[c], using 3 $NiSO_4$ concentrations, by type of drinking water during Ni exposure

Drinking water	Ni exposure	Animals in group	Mean (±SE) cpm, by contents of test well[c]				
				$NiSO_4$ concentrations			
			PHA-M[d] controls	5.40×10^{-6} M	2.70×10^{-6} M	1.08×10^{-6} M	Media controls
500 μg $ZnSO_4$/ml DDE[e]	yes	12	10,026 (2819)	433 (36)	472 (42)	484 (67)	383 (43)
250 μg $ZnSO_4$/ml DDD	yes	12	7,037 (1284)	274 (32)	391 (56)	406 (60)	484 (67)
DD (controls)	yes	13	6,725 (1382)	501 (87)	539 (66)	633 (90)	376 (57)
tap water (controls)	no	13	4,414 (1172)	265 (37)	354 (43)	382 (53)	614 (79)

[a] cpm's are arithmetic means of quadruplicate determinations (58.9% efficiency) for each animal; 5-day culture included a terminal 18-h spike of 0.5 μCi ^{3}H-methyl-thymidine per well.

[b] Hartley stock, non-pregnant; 467.5 ± 51.4 g ($\bar{x}\pm$SE) at beginning of sensitization.

[c] 1×10^5 viable lymphocytes per well; flat-bottomed microtiter plates; RPMI 1640 medium; 10% inactivated fetal bovine serum, 37°C and 5% humidified CO_2.

[d] 0.5 μg mucophytohemagglutinin (PHA-M) in 100 μl medium.

[e] DDD = double-distilled deionized water, solvent for $ZnSO_4$ dietary supplement.

Table 2. Proportion of female guinea pigs[a] with a specific stimulation index[b] (SI) for $NiSO_4$ in the lymphocyte transformation test[c] by type of drinking water supplied 4 weeks before and during the $NiSO_4$ sensitization and challenge protocol[d]

Drinking water	Ni exposure	Number tested	SI equal to or greater than							
			1.50	1.75	2.00	2.25	2.50	2.75	3.00	3.25
500 μg $ZnSO_4$/ml DDE[e]	yes	12	0.58	0.33	0.25	0.17	0.17	0.08		
250 μg $ZnSO_4$/ml DDD	yes	12	0.17	0.08	0.08					
DDD (controls)	yes	13	0.46	0.46	0.39	0.39	0.23	0.15	0.15	0.08
Tap water (controls)	no	13	0.15							

[a] Hartley stock, non-pregnant; 467.5 ± 51.4 g ($\bar{x}\pm$SE) at beginning of sensitization.

[b] $$SI = \frac{\text{allergen well CPM-background CPM}}{\text{control well CPM-background CPM}}$$

[c] 1×10^5 lymphocytes per well in 96-well flat-bottomed microtiter plates; RPMI 1640 media, 10% fetal bovine serum, 37°C and 5% humidified CO_2, 5-day incubation period, including terminal 18 h with 0.5 μCi ^{3}H-methyl-thymidine. $NiSO_4$ concentrations in wells were 5.4×10^{-6} M, 2.70×10^{-6} M and 1.08×10^{-6} M.

[d] Guinea pig maximization test (10).

[e] DDD = double-distilled deionized water, solvent for added metal salts.

exposure, on subsequent lymphocyte transformation by $NiSO_4$ are shown in Tables 1, 2. The mean counts per minute (cpm) for all 3 concentrations of $NiSO_4$ used in the lymphocyte transformation test were lower for both the 250 μg and the 500 μg $ZnSO_4$ treatment groups than for the Ni-exposed controls (Table 1). Using SIs ≥ 1.5 to compare Ni-exposed treatment groups to the Ni-exposed controls (DDD), the group consuming 250 μg $ZnSO_4$/ml DDD had significantly lower SIs ($p = 0.025$) than the Ni-exposed controls (Table 2). The SIs for guinea pigs consuming 500 μg $ZnSO_4$/ml DDD were consistently lower than those for the Ni-exposed controls; however, this difference was not statistically significant.

Intradermal hypersensitivity test

Saline control and 0.03125% $NiSO_4$ $6H_2O$ intradermal test sites were rarely measurable; i.e., 46 of 50 (92.0%) saline sites and 32 of 50 (64.0%) of the 0.03125% $NiSO_4$ sites showed

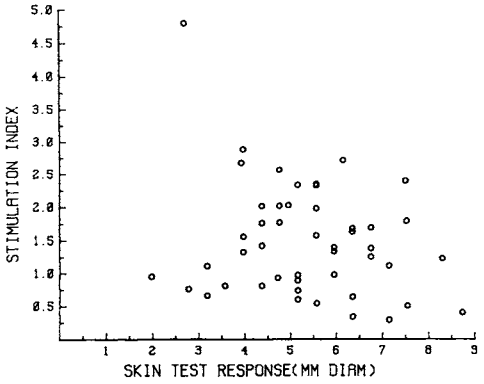

Fig. 2. Peripheral lymphocyte stimulation index by diameter (mm) of skin test response for nickel-exposed guinea pigs.

no reaction at 48 h. Of the sites that could be measured, there were no consistent patterns or trends within or between treatment and control groups.

The largest intradermal reaction in animals exposed to Ni was 8.3 mm in diameter. The largest reaction site in a non-exposed control animal was 7.5 mm. Mean diameters (mm $\pm$ SD) of the largest intradermal reactions, by treatment or control group, were: 5.1 ± 1.6, 500 μg $ZnSO_4$/ml; 5.4 ± 1.4, 250 μg $ZnSO_4$/ml; 5.7 ± 1.2, tap water; and 6.1 ± 1.4, DDD. There was no significant correlation between diameter of intradermal reaction and lymphocyte response in the same animal exposed to Ni (Fig. 2).

Zinc concentrations in skin and blood

The differences in Zn levels of skin or blood between the control and two treatment groups exposed to $NiSO_4$ (Table 3) were not statistically significant. Skin and blood Zn concentrations in sensitized and nonsensitized animals were inconsistent. When these concentrations were plotted for individual Ni-sensitive and non-sensitive guinea pigs, there was no significant correlation between skin and blood Zn.

Discussion

The modified GPMT protocol used in this study provided in vitro evidence of Ni ACD in the Ni-exposed guinea pigs. Based on the LTT results, the SIs of the Ni-exposed controls receiving no Zn in their drinking water, were higher than those of the non-exposed controls.

Zinc supplementation in the drinking water from 4 weeks prior to Ni exposure through the challenge phase had a significant affect on lymphocyte response as measured in the LTT.

Table 3. Zinc content[a] of skin and whole blood of female guinea pigs[b], by type of drinking water consumed during dermal exposure to $NiSO_4$[c] and by sensitivity status[d]

			Mean Zn content			
			skin		blood	
Drinking water	Ni exposure	Status of sensitivity	no.	(μg/g)	no.	(μg/ml)
500 μg $ZnSO_4$/ml DDD (double-distilled, deionized water)	yes	not sensitive	4	20.86	5	3.87
		sensitive	2	25.05	2	3.68
250 μg $ZnSO_4$/ml DDD	yes	not sensitive	6	21.46	5	4.24
		sensitive	1	16.89	1	4.05
DDD controls	yes	not sensitive	8	22.28	7	4.09
		sensitive	3	19.02	2	3.94

[a] Flame AA spectrophotometry.
[b] Hartley stock, non-pregnant; individually caged.
[c] Guinea pig maximization test (10).
[d] Sensitivity to $NiSO_4$ defined by stimulation index ≥ 2.0 in the LTT.

Although only the 250 μg $ZnSO_4$/ml DDD group had significantly lower SIs than the appropriate controls, both the 250 μg and 500 μg groups were consistently lower. Therefore, dietary Zn supplementation should be explored further as a regimen for prevention of Ni ACD in other species including man.

The levels of Zn used in this study did not alter Zn homeostasis, as reflected by Zn concentrations in skin and blood. Aughey et al. (35) found no significant difference in the Zn content of liver, skin, or spleen between mice receiving distilled water or water with 0.5 gm Zn/l (in the form of $ZnSO_4$) over a 6-month period. However, in that study 0.5 g Zn per liter of drinking water did increase plasma Zn significantly. Several authors agree that the Zn content of most tissues, excluding plasma, can only be significantly increased by large Zn supplements if Zn nutriture is initially marginal or deficient (39–41). Future studies which evaluate the effects of Zn on metal-induced ACD could employ Zn-sufficient and Zn-deficient diets. Other metals (copper, magnesium, and manganese) should also be investigated in regard to probable interactions that could block Ni and other important industrial metals (e.g., beryllium, chromium, or cobalt) that are known to induce the ACD syndrome in humans.

Stimulation indices have been reported to be more quantitative and objective measurements of delayed hypersensitivity than either the intradermal or patch test challenges (11, 42). In the present study, 64% of the lowest $NiSO_4$ concentration intradermal challenge sites in previously Ni-exposed guinea pigs had no observable reaction at 48 h. No consistent pattern within or between treatment groups was observed. In a study using only epicutaneous sensitization with $NiSO_4$ and 48 h closed skin patch containing 0.5 ml 2% $NiSO_4$ in distilled water, two groups of guinea pigs had sensitization rates of 63% and 80% based on macroscopic examination of the challenge site 6 h after removal of the bandage (43). Therefore, in future studies the skin patch challenge may be more reliable than the intradermal challenge as an in vivo measure of ACD and a basis for evaluation of the LTT.

The absence of a correlation between in vitro (LTT) and in vivo (intradermal site) responses in the Ni-exposed guinea pigs is consistent with the work of Al-Tawil et al. (44), who reported no correlation or very weak correlations between LTT and patch test responses in humans with clinical Ni ACD. Research to determine influencing factors and kinetics in the in vitro expression of ACD is needed (45). Supplementing in vitro assays of ACD with Langerhans cells might increase the sensitivity and specificity. Much research is now being focused on this cell and its role in cutaneous immune responses (46–48). One recent electron-microscopic investigation found that these cells were not damaged in contact dermatitis in humans (49).

Inter-study variation in sensitization routes, sensitization dosage, use of adjuvants and time of exposure affects comparisons of challenge results. In contrast to human clinical Ni ACD cases which often develop after several years of exposure, these guinea pigs were exposed for only 23 days. Thus, future studies of this type should include multiple periods or longer periods of animal exposure to $NiSO_4$. More attention should be given to induction of Ni sensitization with greater SIs and skin test reactions. The guinea pigs could have been exposed by skin painting, occlusive bandage only, or combinations of these with intradermal exposure using a different adjuvant. Furthermore, there may be a strain of guinea pig that is more sensitive to Ni than the outbred Hartley stock.

Nickel sulfate concentrations of 2.70×10^{-5} and 1.08×10^{-5} were considered inhibiting or toxic to guinea pig peripheral blood lymphocytes (PBL) during 5-day LTT assays. The trypan blue dye-exclusion test might have provided evidence of direct toxicity to PBL; however, it was not performed. Nordlind (50) found that 10^{-3} and 10^{-4} M concentrations of $NiSO_4$ were inhibiting in in vitro cultures of

unsensitized guinea pig lymphoid cells at 5 and 48 h. Both of these observations are consistent with other findings of Ni toxicity. For example, it was shown that 10 μg Ni^{++}/ml produced cytological changes, especially altered nuclear morphology, in rat myoblast cultures (51), while nickel sulfate transformed and reduced the cloning efficiency of hamster embryo cells (52). Several nickel compounds transformed baby hamster kidney cells in vitro (53), and $NiCl_2$ transiently suppressed T-cell mediated immunity in mice (54).

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