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SODIUM LAURYL SULFATE ENHANCES NICKEL PENETRATION THROUGH GUINEA-PIG SKIN. STUDIES WITH ENERGY DISPERSIVE X-RAY MICROANALYSIS

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Abstract

The effect of sodium lauryl sulphate (SLS), a common ingredient of detergents, on the penetration of nickel through the stratum corneum in the guinea-pig skin model was studied with energy dispersive X-ray microanalysis (EDX) to evaluate the barrier-damaging properties of this common detergent. The EDX technique allows a simultaneous determination of physiologically important elements, e.g., Na, Mg, P, Cl, K, Ca and S in addition to Ni at each point of measurement in epidermal cell strata. Our results show that SLS reduces the barrier function to Ni-ion penetration of the stratum corneum. In addition we have shown that EDX allows analysis of the influence of different factors involved in nickel penetration through the skin by giving data on the physiological effects on the epidermal cells caused by the applied substances.

Key Words: Ni-ion, Sodium lauryl sulphate, skin barrier, skin penetration, guinea-pig.

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Introduction

Nickel constitutes one of the most important allergens in present day western environments. In women, nickel is a dominating cause of skin contact allergy. Contact allergic eczema is a so called delayed type of hypersensitivity (type IV reaction). Such reactions demand an initial phase of sensitization where the organism learns to recognize the antigen (the allergen) as "foreign matter". Once the individual is sensitized subsequent contact with the allergen will elicit a contact allergic reaction at the site of contact. In order to elicit such a reaction the antigen (or incomplete antigen = hapten) must be able to pass through the skin barrier.

At present there is a need for knowledge about the different factors needed for the introduction of the allergen to the organism via the skin. These factors are: 1) the factors involved in the passage of the antigen through the skin barrier, 2) the factors involved in the cellular (Langerhans' cell) recognition of the antigen and the processing of it and 3) the presentation of the processed antigen to lymphocytes with a "memory capacity" which can elicit the "avalanche" of the immunological defense mechanisms.

In addition to a number of comparatively new antigens in the environment such as nickel we also have the further complication of factors that may to a lesser or greater extent change the function of the barrier of the skin , e.g., detergents present in cleaning agents and liquid soaps. The passage of an incomplete antigen, e.g., nickel ions, through the skin barrier may thus be modified by the presence of detergents. In dermatological research SLS has been used extensively as a model substance for detergent interaction with skin, hence our choice of detergent.

In dermatological research on skin metal allergies quantitative elemental analysis requires methods which are sensitive down to concentration levels of 100 ppm or better. The energy dispersive X-ray microanalysis (EDX) technique allows simultaneous determination of elements present in the spot of analysis down to concentrations of 100 ppm (1,3). Recently an attempt to determine the Ni content of positive test reactions in

Ni-sensitized individuals was undertaken using this technique (2). However, the amount of Ni was below the detection limit for the method although clearcut positive test reactions were observed. The conclusion was that the concentration level of Ni necessary to elicit an allergic contact reaction may be very low. This contention gains support from preliminary in vitro results of Ni penetration through human skin using PIXE (proton induced X-ray emission) analysis sensitive down to concentration levels of approximately 10 ppm (4).

The aim of the present paper is to present data obtained by means of energy dispersive X-ray microanalysis (EDX) on the penetration of nickel (Ni^{2+}) ions through SLS-treated guinea pig skin.

Materials and Methods

Female albino guinea-pigs weighing 500-1000 g were shaved on the back. Subsequently vessels were glued to the shaved skin surfaces. The vessels were filled with either 5% $NiSO_4$ in distilled water or 5% $NiSO_4$ + 5% sodium lauryl sulfate (SLS) solution. Three groups of animals were used. One group (n=3) was exposed to the plain $NiSO_4$ solution, a second group (n=2) to the $NiSO_4$ + 5% SLS solution and the third group (n=3)served as control. The animals were killed with barbiturate and tissue samples were taken for conventional light microscopy and for EDX analysis. The latter tissue samples were quench frozen and stored in liquid nitrogen until sectioned on a cryostat at $-20^{o}C$ to $-30^{o}C$ to a section thickness of 15 µm. The sections were collected on a carbon specimen holder and freeze-dried over-night in the cryostate (6).

EDX analysis was performed on a JEOL 1200 EX scanning transmission electron microscope fitted with a Tracor energy dispersive X-ray analysis equipment. Physiologically important elements, i.e., Na, Mg, P, S, Cl, K and Ca were analysed at two levels of the epidermis, the stratum spinosum and the stratum germinativum.At each level of analysis four different intracellular locations were chosen. Absolute quantitation of the elemental content was obtained by using a standard (c.f. 5). Ni was analysed at four levels of the skin, stratum corneum, stratum spinosum, stratum germinativum and in the papillary dermis. In the case of the Ni content only a qualitative determination was performed.

Students' t-test was used to compare the elemental content of the exposed skin to that of the controls.

Results

The light microscopic findings are illustrated in figs. 1-3. After exposure to the plain Ni-solution mild dermal and epidermal edema was observed. Exposure to the Ni+SLS solution resulted in marked changes in the keratinocytes consisting of shrunken cells and cell nuclei and occasional losses of cell contacts. The effect of the Ni+SLS exposure was conspicuous in the stratum corneum where the corneocytes had a compacted rather than a swollen appearance (Fig 3).

Table 1 The elemental content of stratum germinativum (GER) and stratum spinosum (SP) in three groups of guinea-pigs given as mmol/kg dry weight (mean and standard deviation)* = $p < 0.05$ compared with controls

Stratum Germinativum

	Na	Mg	P	S	Cl	K	Ca
Control n=3	166 (29)	21 (1)	557 (83)	367 (42)	290 (18)	259 (20)	13 (2)
Nickel n=3	199 (57)	31 (11)	657 (116)	349 (43)	325 (54)	299 (35)	21* (2)
Nickel + SLS n=2	135 (80)	13* (6)	478 (12)	331 (10)	296 (15)	214 (33)	25* (2)

Stratum Spinosum

	Na	Mg	P	S	Cl	K	Ca
Control n=3	99 (33)	28 (2)	570 (59)	381 (39)	347 (49)	287 (51)	17 (4)
Nickel n=3	135 (14)	30 (8)	515 (166)	312 (111)	349 (45)	267 (59)	22 (3)
Nickel+ SLS n=2	120 (80)	12* (10)	354* (60)	373 (67)	325 (62)	195* (13)	21 (4)

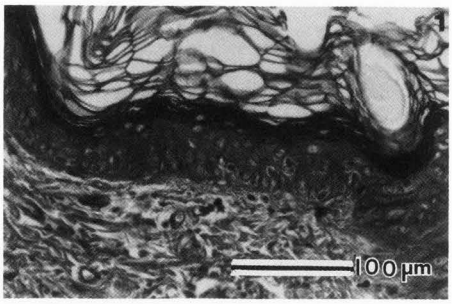
Table 2. Relative Ni content of different strata of guinea-pig epidermis after exposure to nickel sulfate in water or in 5% SLS.

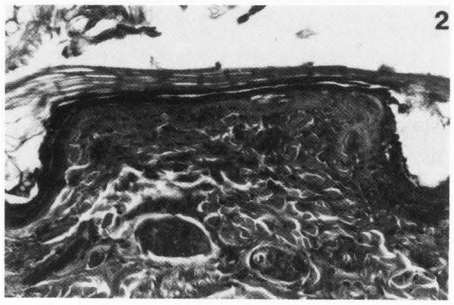
Group	St corn	str spin	str germ	dermis
Control	–	–	–	–
$NiSO_4$	+	–	–	–
$NiSO_4$/SLS	+	+	+	+

The results of the X-ray microanalysis is given in Table 1 (the elemental content of the epidermis) and in Table 2 (the nickel analysis). Compared with the controls there was a significant reduction of Mg, P, and K in the epidermis exposed to the Ni+SLS-solution. The calcium content was increased after exposure to both solutions. No significant changes in the elemental content was found in epidermis after exposure to the Ni-solution in this limited study.

Discussion

Little is presently known about the details of allergen penetration through the skin barrier in general and in particular with relation to the complex situation presented by simultaneous exposure of the skin to detergents. In contrast to





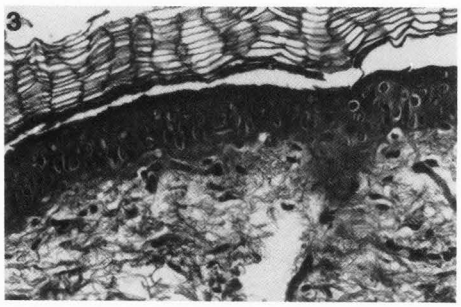


Fig. 1. Normal skin, non-exposed.

Fig. 2. Skin exposed to Ni-solution. Note the dermal edema and increased perinuclear vacuolisation in epidermis.

Fig. 3. Skin exposed to Ni+SLS-solution. Note the compact epidermis with shrunken cells and the compacted stratum corneum which is in contrast to the swelling observed in the stratum corneum in Figs.1 & 2.

allergens consisting of organic molecules, the metal ions with an allergy provoking potential can be detected by sensitive particle probes in inertly prepared specimens. The great advantage of the particle probes (EDX and PIXE) is that a **simultaneous** recording of a great number of interesting elements is possible down to a compartment size of cell or even smaller. We are thus able to get information on the physiological status of the cells exposed to the influence of the "invading" ion at the same time as we can assess the amount of that particular ion. Thus effects such as cell damage or growth stimulation may be recorded. The EDX has a spatial resolution which allows elemental detection at subcellular levels with a sensitivity down to 100 ppm (parts per million) in contrast to the PIXE which at present allows a spatial resolution at a cellular level but with a sensitivity down to better that 10 ppm.

In the present investigation SLS is a detergent which exercises its influence directly on the membrane structures of the stratum corneum, i.e., the diffusion barrier of the skin. In the SLS exposed skin we recorded an enhanced Ni penetration compared to Ni in plain water and detectable amounts of Ni were found as deep down in the skin as the dermis. The combined effect of SLS and Ni on the epidermal cells resulted in detectable changes in the elemental content of the metabolically active epidermal cells. In parallel to the observations on the effect of the plain Ni solution we found a profile of low Mg, P and K and increased Ca values which indicates cellular damage in the stratum germinativum and spinosum of the epidermis (4), a finding in accordance with the light microscopic observations.

On exposure to the plain $NiSO_4$ solution Ni is only detectable in the stratum corneum, i.e. the Ni amounts to more than 100 ppm. It is pertinent to point out that this does not exclude the possibility of concentration levels of Ni below 100 ppm throughout the skin cross section.

At present it appears that Ni presented to the skin in ionized form penetrates the barrier in very low concentrations, probably <20 ppm under non-experimental conditions (1). This means that **very low** Ni amounts will elicit an allergic reaction once the individual is sensitized. The EDX method generally is not sensitive enough to allow the concentration profile of the penetrating Ni ion to be revealed in an inertly prepared specimen. However, the present study shows that the conspicuous increase in penetration caused by the presence of an anionic detergent (SLS) allows at least a semiquantitative analysis of such a profile down to the dermis. The advantage of a simultaneous analysis of physiologically important parameters, such as changes in Na, K, of the epidermal cells underlines the value of EDX analysis in the study of the skin barrier properties under various conditions. An extension of this work including a study of the effect of cationic and non-ionized detergents on Ni penetration through the skin is obviously of great interest and is presently underway.

Acknowledgements

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Discussion with reviewers

C. Ward Kisher: Do you believe that SLS acts to increase membrane permeability to Ni in a selective way or to a spectrum of metals? Or do you believe the action of SLS is through a different mode?
Authors: The amphiphilic character of SLS makes it very likely to interfere with any integral proteins of cell membranes even to the extent that such proteins may be solubilized. This will of course create an imbalance in the cellular metabolism and ionic transmembrane exchange which may be one of the explanations for the cell damage observed. In the stratum corneum SLS may interfere with the intercellular lipids and the lipid part of the corneocytes. It may possibly even solubilize some of that lipid material creating "holes" , i.e. a looser lateral packing of the lipid units, which will increase the volume of the "water channel" (c.f. 8). Such an effect is expected to open the way to the penetration of water soluble substances of all kinds. At present we are ignorant of the possible structural effects that detergents have **in situ** on the keratin macromolecules. However, a conspicuous swelling of keratin occurs when sheets of stratum corneum is immersed in SLS solutions (Forslind, B. unpublished observations). We thus have good reasons to believe that the "water channel" volume is increased also due to this swelling effect.

C. Ward Kischer: How do you account for the Ni being found in the dermis?
Authors: It appears that Ni-binding to organic material has a low stablity (7) and that carboxyl groups and amino acids are mainly involved. It is thus permissible that in the span of a comparatively long time, 18 - 24 hours in our present investigation, Ni ions may well diffuse down to the dermis.

R. Warner: In the data of Table 2, what was the criteria for Ni detection ?
Authors: Since the amounts of Ni in normal skin is below the detection limit of our EDX system we took a qualitative approach to the problem. Hence, the presence of Ni has been indicated where the Ni peak unambiguously overlays the background (roughly characteristic/continuum counts > 1/1).

R. Warner: In the control guinea-pigs as presented in Table I, K is considerably lower and Cl considerably higher than that reported in earlier studies (e.g. Lindberg et al., 1983), although Na levels are quite comparable. What would account for such large changes in intracellular ionic content ?
Authors: At present we have no single explanation for this incongruence in our K-values. One factor that may be responsible is the fact that in this study new equipment was used. The image from the specimen is considerably better and allows an unequivocal distinction of the stratum germinativum and dermis. In our previous studies this was not always clear and measurements with low K and high Cl in the stratum germinativum were viewed with suspicion. This has probably introduced a bias towards cells with high K and low Cl. Whether cells with a low K in the stratum germinativum represent a separate population or whether these cells are damaged at some step of the preparation procedure remains to be investigated.

R. Warner: In earlier studies on Ni effects on the guinea-pig skin (authors' reference 3), Ni was shown to statistically reduce K concentrations. In this paper it is stated to increase K. Can this discrepancy be explained ?
Authors: The effect of Ni-solutions on metabolically active epidermal cells appears to be dose and time dependent. This implies that the response of the cells to Ni exposure can cause an increased mitotic activity (K ↑) or a toxic injury (K ↓).

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