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Elemental Changes in Guinea Pig Epidermis at Repeated Exposure to Sodium Lauryl Sulfate

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Epidermal hyperplasia is the response of the epidermis to external harmful stimuli. The control and regulation of this hyperplasia is not completely understood. It has been proposed that changes in the cellular sodium/potassium ratio are of importance in the regulation of cell proliferation. To evaluate if such a change in the elemental content of epidermal cells can be one factor to consider at irritant contact dermatitis, we performed a quantitative assessment of sodium lauryl sulfate (SLS)-induced contact reactions in the guinea pig. SLS was applied 1, 2 or 3 times and biopsies were obtained at 24 and 84 h after the last application. It was found that repeated exposures to SLS induced a hyperplasia of epidermis at 24 h persisting at 84 h. At 24 h there were significant changes in the sodium and potassium content of the keratinocytes. At 84 h there was still an increased potassium level in the cells and the sodium/potassium ratio was significantly decreased in epidermis exposed three times to SLS. This implies that changes in cellular sodium/potassium ratios occur in epidermal hyperplasia following irritant stimuli. ***Key words: Irritant contact dermatitis; Hyperplasia; Light microscopy; Energy dispersive X-ray microanalysis (EDX).***

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Epidermal hyperplasia is a non-specific response of the skin to external irritant stimuli (1). A hyperplastic growth is seen in the epidermal response at irritant contact reactions as well as at the initiation of neoplasia (2,3). The occurrence of contact dermatitis is a growing problem in dermatology (4). One major group of contact dermatitis is the so-called traumiterative contact dermatitis (5,6) where repeated exposures to lowgrade irritant stimuli induce an eczematous reaction. The development of such an irritant reaction is not only dependent on the dose of the irritant but also on the frequency of the contact and other factors such as the actual condition of the exposed skin. One major group of substances known to induce this type of irritant reactions is detergents. In order to obtain a better understanding of the mechanisms controlling the epidermal response to irritants we use energy dispersive X-ray microanalysis (EDX) to determine elemental changes in the epidermal keratinocytes at irritant reactions (7). This type of analysis reveals information about the functional state of the cells (stimulation, cell damage, and cell death) mirrored by the cellular content of sodium, magnesium, phosphorus, sulphur, chloride, potassium, and calcium (8–12). Using the EDX technique we have previously shown that a single application of sodium lauryl sulfate (SLS) induces transient membrane damage followed by an increased proliferation of keratinocytes (13). We have also demonstrated that the epidermal hyperplasia following a single application of n-hexadecane is preceeded by elemental changes in the keratinocytes compatible with a cell membrane damage (6–24 h following exposure) (7). The induced hyperplasia is associated with a simultaneous shift in the epidermal sodium-potassium relation with a low ratio (7,14). The sodium-potassium ratio is considered a possible regulatory mechanism in the control of cell proliferation (15). It is tentatively suggested that the change in sodium-potassium ratio in the epidermis during hyperplasia may play a part in controlling the keratinocyte proliferation. In the present study we have for the first time determined the elemental changes in guinea-pig epidermis after repeated exposures to SLS in order to further investigate this possibility.

MATERIAL AND METHODS

Animals

The backs of adult female guinea-pigs were clipped with an electric clipper prior to application of the test substance. The animals were killed by an intraperitoneal overdose of barbiturate prior to tissue sampling.

Test substance

A 5% sodium lauryl sulfate (SLS) water solution was used. Five drops of the solution was applied to an area of 2×2 cm on the clipped back. The solution was spread over the test area with a cotton swab for 15 sec. The procedure was repeated twice in immediate sequence.

Experimental procedure

Two groups of animals were used. In group 1 (5 animals) the SLS solution was applied to three different skin areas, one, two or three times respectively, with 24 h between applications. Skin specimens were taken 24 h after the applications. In group 2 (4 animals) the solution was applied to two different skin areas one or three times respectively with 24 h between applications. Skin samples were taken 84 h after the applications. Controls were taken from non-exposed skin on each animal. Specimens were taken for light microscopy evaluation and for energy dispersive X-ray microanalysis.

Energy dispersive X-ray microanalysis (EDX)

The skin specimens were divided, snap-frozen and stored in liquid nitrogen. Freeze-sections were cut in a cryotome at −20° to −30°C to a section thickness of 12–16 µm. The sections were collected on carbon

Table I. *The epidermal thickness in arbitrary units following 1, 2 or 3 applications of SLS (group 1, skin specimens at 24 h after last application) and following 1 or 3 applications of SLS (group 2, skin specimens at 84 h after last application) to the skin surface*

		Number of applications			
		Control	1	2	3
Group 1 $n=5$	Mean	15.1	17.4	24.1	28.7
	s.d.	1.6	1.9	2.7	2.2
Group 2 $n=4$	Mean	13.7	27.7	–	25.6
	s.d.	1.9	1.6		1.7

specimen holders and were freeze-dried in the cryostate overnight (16). EDX-analysis was performed on a JEOL 1200 TEMSCAN fitted with a Tracor 5500 energy dispersive X-ray system. Physiologically important elements, i.e., Na, Mg, P, S, Cl, K, and Ca were analysed at two levels of the epidermis, the stratum germinativum and the stratum spinosum. At each level of analysis, four different locations were chosen. Absolute quantitation of the elemental content was obtained by using a standard (17,18).

Light microscopy

The specimens were fixed in buffered formaldehyde, embedded in paraffin-wax and the sections were stained with haematoxylin-eosin. At a magnification of ×400, the epidermal thickness (from the basal membrane to the stratum corneum) was measured using an ocular grid with arbitrary units. At least 20 measurements were made per sample.

Statistical analysis

A mean of the elemental content of epidermis was calculated using the results of the stratum germinativum and the stratum spinosum This mean elemental content of epidermis was submitted to a variance analysis. The analysis was performed for each element in both groups. Furthermore, in group 2 we also performed a *t*-test comparing the controls with the epidermis exposed three times to SLS.

RESULTS

The application of SLS, one, two or three times, resulted in skin reactions with erythema. The reactions were more pronounced after two and three applications and the skin showed a slight scaling after three treatments. In the 84 h group the skin showed palpable oedema and scaling but no erythema. The epidermal thickness increased with an augmenting number of treatments in group 1 and was still increased at 84 h (Table I). The result of the EDX-analysis 24 h after applications of SLS (group 2) is given in Table II. It was found that the elemental content of sodium and potassium was increased after repeated exposure, whereas the sodium-potassium ratio had a tendency towards but was not significantly decreased.

The result of the EDX-analysis in group 2 (84 h after application) showed a significant increase in the epidermal potassium content whereas the other elements were not significantly

Table II. *The results of the EDX-analysis for group 1 with 1, 2 or 3 applications of SLS and skin specimens taken at 24 h after last application*

Elemental content in mmol/kg dry weight is given with mean and (s.d.), $n=4$.

Element		Number of applications of SLS				Variance analysis for G+S
		Control	1	2	3	
Na	G	102 (26)	126 (22)	164 (12)	150 (23)	
	S	95 (25)	118 (16)	161 (11)	125 (25)	
	G+S	98 (24)	122 (18)	163 (10)	137 (23)	$p<0.05$
Mg	G	14 (6)	17 (4)	14 (5)	16 (4)	
	S	12 (3)	15 (7)	11 (4)	15 (4)	
	G+S	13 (5)	16 (3)	12 (4)	15 (3)	N.S.
P	G	369 (77)	403 (56)	424 (50)	431 (65)	
	S	305 (56)	367 (56)	400 (78)	328 (93)	
	G+S	337 (58)	390 (46)	412 (61)	379 (78)	N.S.
S	G	215 (53)	223 (39)	210 (51)	202 (16)	
	S	211 (38)	232 (26)	239 (42)	196 (31)	
	G+S	213 (44)	228 (28)	224 (46)	199 (22)	N.S.
Cl	G	171 (63)	192 (25)	237 (52)	247 (47)	
	S	182 (79)	201 (33)	251 (53)	212 (61)	
	G+S	176 (68)	196 (25)	244 (42)	228 (54)	N.S.
K	G	128 (52)	164 (28)	197 (37)	242 (64)	
	S	118 (46)	168 (33)	217 (60)	196 (62)	
	G+S	123 (48)	166 (28)	207 (48)	219 (62)	$p<0.05$
Ca	G	18 (3)	15 (3)	18 (11)	15 (3)	
	S	21 (6)	16 (3)	23 (8)	16 (7)	
	G+S	19 (4)	16 (2)	22 (8)	16 (4)	N.S.
Na/K ×100	G	88 (23)	79 (19)	85 (11)	67 (23)	
	S	90 (27)	72 (15)	78 (15)	69 (21)	
	G+S	89 (24)	75 (16)	82 (13)	68 (22)	N.S.

G = stratum germinativum; S = stratum spinosum; G+S = mean of G and S; N.S. = not significant ($p>0.05$).

Table III. *The results of the EDX-analysis for group 2 with 1 or 3 applications of SLS and skin specimens taken at 84 h after last application*

Elemental content in mmol/kg dry weight is given with mean and (s.d.), $n = 4$.

Element		Number of applications of SLS			Variance analysis for G+S
		Control	1	3	
Na	G	138 (33)	138 (37)	156 (25)	
	S	129 (37)	124 (20)	132 (20)	
	G+S	134 (35)	131 (28)	144 (23)	N.S.
Mg	G	13 (2)	17 (2)	20 (6)	
	S	17 (4)	18 (4)	23 (3)	
	G+S	15 (2)	17 (3)	22 (4)	N.S.
P	G	441 (69)	375 (37)	491 (49)	
	S	358 (72)	353 (63)	393 (54)	
	G+S	400 (48)	364 (49)	442 (49)	N.S.
S	G	253 (58)	211 (33)	236 (33)	
	S	234 (22)	228 (28)	250 (22)	
	G+S	244 (28)	219 (29)	243 (23)	N.S.
Cl	G	206 (62)	180 (50)	234 (47)	
	S	185 (40)	167 (38)	222 (46)	
	G+S	196 (46)	174 (43)	228 (46)	N.S.
K	G	166 (24)	191 (43)	293 (32)	
	S	148 (38)	170 (31)	272 (33)	
	G+S	157 (27)	180 (35)	283 (31)*	$p < 0.05$
Ca	G	18 (6)	16 (2)	14 (1)	
	S	14 (3)	13 (2)	13 (3)	
	G+S	16 (4)	15 (2)	14 (2)	N.S.
Na/K	G	83 (18)	72 (12)	53 (5)	
×100	S	90 (20)	74 (12)	48 (2)	N.S.
	G+S	87 (17)	73 (11)	51 (4) *	$p = 0.06$

G = stratum germinativum; S = stratum spinosum; G+S = mean of G and S; N.S. = not significant ($p > 0.05$); * = $p < 0.05$ t-test comparing 3 applications with control.

altered (Table III). The sodium-potassium ratio was significantly decreased at 84 h after 3 SLS exposures compared with the control biopsies ($p < 0.05$; *t*-test).

DISCUSSION

In the present study it is demonstrated that the repeated application of a detergent (sodium lauryl sulfate) to the skin induces an epidermal hyperplasia associated with an increase in the cellular sodium and potassium contents. The hyperplasia is still present 3.5 days after the last application with a persistent increase in the potassium levels and a decreased sodium-potassium ratio. The mechanisms regulating and controlling cell-proliferation are not fully understood. It is known that rapidly dividing cell populations are characterized by a high sodium-potassium ratio, whereas cell systems with low mitotic activity have low sodium-potassium ratios (9,11,12, 19,20,21). Transient increases in the sodium-potassium ratio can induce mitosis (22) in cultured cells. Thus it is possible that variations in the relation between sodium and potassium in the epidermal cells can be of importance for the regulation of cell proliferation in the epidermis. It has previously been demonstrated that a single application of SLS induces elemental changes compatible with membrane damage followed by an increase in keratinocyte proliferation (13). By analysing thick sections with the EDX technique, it has also been shown that a single application of n-hexadecane to the skin induces a transient hyperplasia of the epidermis associated with a decreased sodium-potassium ratio (14). This decrease preceeded the normalization of the epidermal thickness. Comparing those findings with the results of the present study shows that the elemental changes found in groups 1 and 2 reveal a similar pattern. At 84 h after application of SLS there was still an increased epidermal thickness with a simultaneous decrease in the sodium-potassium ratio, depending on an increased potassium level. The other elements show no obvious alterations at 84 h.

In epidermal hyperplasia there is an increased density of ribosomes (2,3) and it is probable that a major part of the increased cellular content of potassium is associated to this subcellular fraction. In a previous study it was shown that a single exposure to n-hexadecane results in an initial increase followed by a decrease in sodium (14). We have also shown that a single exposure to SLS induces a transient increase in the cellular sodium content (13). These findings suggest that membrane damage occurs, rendering the cell open to ion diffusion which leads to an increase in the cellular sodium. The explanation for the findings in the present study of an increased sodium content in group 1 after SLS exposure then probably represents an effect of repeated insults to the cell membranes. In the group of animals sacrificed 84 h after the last SLS-application the sodium content appears normal, a fact which suggests that the damage is transient.

In conclusion, it is demonstrated that detergent-induced hyperplasia is associated with a decreased sodium-potassium ratio depending on a persistent increase in potassium. This change in sodium-potassium relation is probably a non-specific response at epidermal hyperplasia and is thought to depend on the increase in nucleic acids found in the keratinocytes at hyperplasia (2,3).

We have also demonstrated that the EDX technique provides an efficient tool for the evaluation of functional alterations in the epidermal keratinocytes following single or repeated exposure to irritant stimuli.

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