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Changes in Sodium–Potassium Ratio in Guinea Pig Epidermis in *n*-Hexadecane-induced Hyperplasia

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A transient epidermal hyperplasia was induced in guinea pig epidermis by a single application of *n*-hexadecane. The epidermal response was analysed by light microscopy and by energy dispersive X-ray microanalysis (EDX). The epidermal hyperplasia reached a maximum between 96 and 192 h after the application. The hyperplastic response was associated with a depressed sodium–potassium ratio (increased potassium, decreased sodium) in the keratinocytes at 96 h, beginning already at 48 h. At 24 h there were no major differences in elemental content, compared the controls. The result of the present study is consistent with the hypothesis that alterations in the functional state of the epidermal keratinocytes are associated with changes in the sodium–potassium ratio in the cells. The absence of major elemental changes at 24 h indicates that the initiation of the hyperplastic response occurred prior to this time point. ***Key words: Energy dispersive X-ray microanalysis, Cell proliferation, Irritant reaction, Elemental changes***

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The control and regulation of cell and tissue growth is a central question for the understanding of cell kinetics under normal and pathological conditions. In 1971, Cone (1) presented a theory on the mechanism of normal mitotic control and oncogenesis. He postulated that the balance between the resting state and mitosis in cells is regulated by the cell membrane potential which in turn is dependent on the sodium–potassium content of the cells. Under steady-state conditions, a high sodium content and consequently high sodium–potassium ratio would be associated with an increased mitotic activity. This hypothesis has been supported by other authors working with biochemical techniques and fibroblasts in culture (2) and with energy dispersive X-ray microanalysis on normal and neoplastic tissue (3).

It also appears as if transient changes in the cytoplasmic content of sodium might alter and initiate DNA-synthesis and mitotic activity in cells (2). In a previous study (4) on sodium lauryl sulphate induced hyperplasia in guinea pig epidermis, we found that the hyperplastic response was preceded by a transient increase in the sodium content of the keratinocytes. The aim of the present study was to further evaluate elemental changes in the keratinocytes during conditions when the normal epidermal steady state is altered. As a model we have looked at a chemically induced hyperplasia.

MATERIALS AND METHODS

Model system

Female albino guinea pigs weighing approximately 500 g were used. The back was sheared with electric clippers. The animals were killed with an intraperitoneal overdose of a barbiturate prior to biopsy sampling.

Test substance

n-Hexadecane (Sigma Chemicals) was used to induce hyperplasia of the epidermis. Using a pipette, a single dose of 0.5 ml *n*-hexadecane was applied to an area of 3×3 cm on the back.

Experiments

Skin biopsies were taken at 0, 24, 48, 96, and 192 h following the application of *n*-hexadecane. The biopsies were divided and processed for conventional light microscopy and energy dispersive X-ray microanalysis (EDX). Light microscopy was performed at each interval and EDX was done at 0, 24, 48, and 96 h. Four or 5 animals were studied at each interval.

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 stratum corneum) was measured using an ocular grid with arbitrary units. On each biopsy the epidermal thickness was determined as the mean of 10 separate measurements. The number of keratinocyte

Table I. *Epidermal thickness (arbitrary units) and number of cell nuclei along a line peripendicular to the dermal–epidermal junction after a single application of n-hexadecane (Mean ± SD)*

Hours	*n*	Epidermal thickness	Number of cell nuclei
0	4	0.96±0.19	1.34±0.18
24	4	2.03±0.21	1.4±0.25
48	5	2.98±0.3	2.4±0.51
96	5	3.55±0.8*	3.42±0.41*
192	5	1.96±0.27	1.9±0.17

* $p<0.05$.

cell nuclei was determined as the mean of 10 counts of cell nuclei along a grid line perpendicular to the dermal–epidermal junction.

Energy dispersive X-ray microanalysis (EDX)

The biopsies were quench frozen and stored in liquid nitrogen until sectioned on a cryostat at −20°C to −30°C to a section thickness of 12–15 µm. The sections were collected on a carbon specimen holder and freeze-dried overnight in the cryostat (5).

EDX analysis was performed on a JEOL 1200 EX scanning transmission electron microscope fitted with a Tracor energy dispersive X-ray analysis apparatus. Physiologically important elements, i.e. Na, Mg, P, 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 (6).

Statistical analysis

Wilcoxon's rank sum test, two-tailed, was used to compare the epidermal thickness and the number of cell nuclei at 96 h with the controls and to compare the elemental changes at 24, 48, and 96 h vis-à-vis the controls. The analysis on the elemental changes was performed on the mean values of stratum germinativum and stratum spinosum.

RESULTS

The application of *n*-hexadecane resulted in a clearly detectable erythema at 24 h, with the addition of edema at 48 h. At 96 and 192 h after the application the erythema had diminished and was replaced by a scaling of stratum corneum.

The epidermal thickness and number of cell nuclei are given in Table I. It was found that a single application of *n*-hexadecane induced a statistically significant increase ($p<0.05\%$) in both the epidermal thickness and the number of cell nuclei, with a maximum between 96 and 192 h.

The EDX results are given in Table II. The statistical analysis was performed on the mean values of stratum germinativum and stratum spinosum. At 96 h there was a significant decrease ($p<0.05\%$) in the

Table II. *Elemental changes in epidermis after a single application of n-hexadecane (mmol/kg dry weight)*

Statistical analysis was performed on the means of st germinativum and st spinosum

		Element (mean ± SD)						
Hours	No.	Na	Mg	P	Cl	K	Ca	Na/K
St germinativum								
0	4	196±36	21±7	523±67	318±50	271±82	15±4	0.72±0.27
24	4	196±25	18±4	351±52	280±48	260±21	13±4	0.75±0.05
48	5	180±22	28±4	497±96	268±45	329±87	13±4	0.55±0.23
96	5	146±9	27±4	482±45	269±28	404±55	17±9	0.36±0.04
St spinosum								
0	4	174±39	20±6	438±63	309±26	244±38	17±3	0.71±0.32
24	4	178±21	26±3	404±45	292±18	273±27	15±4	0.65±0.07
48	5	151±23	32±3	560±137	334±99	494±190	18±6	0.31±0.15
96	5	128±15	37±2	487±45	277±26	428±40	15±3	0.30±0.03
Statistical analysis vs. 0 hours								
24		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
48		n.s.	*	*	n.s.	n.s.	n.s.	n.s.
96		*	*	n.s.	n.s.	*	n.s.	*

* $p<0.05$.
n.s. = not significant.

sodium level and an increase in the magnesium and the potassium concentrations. The sodium–potassium ratio was thus lowered. These changes were discernible already at 48 h when there also was an increase in the stratum spinosum phosphorous content. There were no major differences in the elemental content of the keratinocytes at 24 h after the application of *n*-hexadecane vis-à-vis controls.

DISCUSSION

It has long been known that the epidermis responds with a hyperplastic growth to harmful chemical stimuli (7, 8). However, the biochemical events triggering and controlling this process are still poorly understood. It is probable that a chemical injury/stimulus alters the phospholipid turnover and modifies the membrane properties of the cells and thereby, among other things, releases mediators acting at the cellular level (9, 10, 11). According to the literature cited in the introduction there is evidence indicating that the transmembranous sodium–potassium relation is one factor of importance in the regulation of the cellular mitotic activity.

With the introduction of EDX in biology (12, 13) it has become possible to study cellular physiology in frozen and freeze-sectioned cells and tissues. The EDX technique allows a detection and quantitation of sodium and heavier elements down to a concentration of 100 ppm. With EDX, as applied in the present study, we obtain a spatial resolution at the level of a keratinocyte (14, 15). Due to the fact that epidermis is a stratified epithelium, we prefer to present our measurements as results per epidermal stratum.

In 1963, Hoekstra & Phillips (16) showed that *n*-hexadecane is a potent inducer of epidermal hyperplasia. The biochemical changes following application of *n*-hexadecane have been studied by several groups. For example, it has been shown that after application there is a rapid fall in cyclic-AMP (17) and an activation of ornithine decarboxylase (18), followed by an increased DNA synthesis with a maximum within 24–48 h (17, 18). In the present investigation we induced a transient epidermal hyperplasia with a single application of *n*-hexadecane. Our light microscopic control confirmed that there was a maximum epidermal thickness in the interval between 96 and 192 h after the stimulus.

The EDX analysis of the elemental changes in the epidermis revealed that there was an increased content of magnesium and potassium and a concomitant decrease in the sodium content at 96 h. These changes were discernible already at 48 h, in association with an increased phosphorous content in stratum spinosum. At 24 h there were no significant differences in these elements vis-à-vis the controls. Increased levels of cellular magnesium, phosphorous, and potassium in combination with high sodium concentrations can be found in tissues with an increased proliferative activity (3). The marked depression of the sodium–potassium ratio at 48 and 96 h, vs. 24 h and controls, is a shift in the sodium–potassium relation that would be more compatible with a low mitotic activity (1). However, it has to be remembered that the hyperplastic response to *n*-hexadecane is a dynamic process and that the hyperplastic epidermis is not in a steady state. There is a possibility that the change in the Na/K ratio reflects the hyperplastic state of the tissue (with a both qualitative and quantitative alteration in the relation between nuclei and cytoplasm) and an accumulation of ribosomes (3, 8). To resolve this question there is a need for EDX analysis of thin sections with a resolution that permits a distinction between the nucleus and the cytoplasm in combination with a morphometric analysis of the volume relations between these sub-cellular components. It can be speculated whether this change in Na/K might in turn influence the proliferative activity in the hyperplastic epidermis, as it preceded the regression of the hyperplasia.

It has previously been shown (cf. 8) that, during the hyperplastic response to chemical stimuli, the development of the hyperplasia is associated with a transient expansion of the proliferative compartment in epidermis with an increased number of dividing supra-basal keratinocytes. With the application of the EDX technique on thick sections, we did detect similar changes in the elemental content of the kertinocytes in both the stratum germinativum and in stratum spinosum. Considering the possibility of an expanded proliferative cell population in combinition with the spatial resolution obtained in our EDX analysis, it is probable that the elemental changes detected in stratum spinosum do reflect changes associated with alterations in the functional state of the keratinocytes. A more unexpected finding was the normal elemental distribution found at 24 h. In our previous work on the epidermal response to sodium lauryl sulphate (4) we found a transient increase in the sodium content at 24 h preceding the hyperplastic response. There are, however, several factors still incompletely known that must be considered. The find-

ings at 24 h indicate that changes in the keratinocytes associated with the induction of the *n*-hexadecane-dependent hyperplasia occurred prior to this time point. It is known that *n*-hexadecane induces a rapid proliferative response (17, 18) and that the signal to an increased mitotic activity might be transient (2). It is thus probable that an initial transient change in the elemental content is not detected at 24 h. Other factors that must be further investigated are differences between substances regarding their mode of action, e.g. point of interaction at the cellular level, time course, and dose effects.

In conclusion, the present study, together with our previous work, shows that alterations in the proliferative activity in the epidermal keratinocyte population are associated with changes in the elemental content of the cells. These elemental changes reflect the functional state of the cells and it is therefore possible to use the EDX technique to study functional alterations in the cells of epidermis under various experimental conditions.

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