

SHORT REPORTS

Cyclic AMP and Psoriasis Once More

Svend Wadskov, Vibeke Kassis
and Jørgen Søndergaard

Department of Dermatology, University of Copenhagen,
Hvidovre Hospital, Copenhagen, Denmark

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Abstract. Imbalanced cyclic nucleotides are implicated as an underlying pathogenetic mechanism in psoriasis, but conflicting data on the levels of cAMP in psoriatic tissue have been obtained by different laboratories. Using heat separation and a competitive protein binding cAMP assay, a noticeably decreased epidermal level of cAMP was detected in newly formed guttate lesions in 10 patients with psoriasis (involved: 4.32 ± 1.06 pmol/mg dry weight mean $\pm$ S.E.M.), uninvolved: 7.97 ± 1.63 pmol/mg dry weight mean $\pm$ S.E.M.). In proliferative rat skin, bidirectional alterations in cAMP levels are known to occur. On the basis of present observations, we assume that the conflicting data hitherto obtained in psoriatic tissue similarly may merely reflect the various levels of cAMP at different developmental stages.

Imbalanced cyclic nucleotides have been claimed to represent a key factor for the abnormal proliferation and differentiation in psoriasis. Increased levels of cGMP in psoriatic lesional tissue have been demonstrated twice by the same group (6).

Conflicting data have been obtained for cAMP by the same and by different groups (4, 5, 6, 10).

In the present communication we report on a definitely decreased level of cAMP in guttate psoriatic lesions.

MATERIAL AND METHODS

Reagents

cAMP assay kits were obtained from the Radiochemical Centre, Amersham (RCA), U.K.

Liquid scintillation counting was performed in a Beckman type CS 250 liquid scintillation counter. Scintillation fluid was Instagel 6003059 from Packard Instruments Company, Ill., USA.

A 1 ml Potter-Elvehjem glass homogenizer was used. The reaction was carried out in 7×11 mm test tubes.

Patients

Biopsy materials were obtained from newly formed guttate psoriatic lesions and from normal-appearing skin at a distance of approximately 1 cm from the psoriatic lesions. Biopsies from 10 psoriatic subjects were examined. Their ages ranged between 24 and 71 years. No topical treatment of the biopsied areas had been given for at least one week and no systemic drugs were administered.

Sampling

Punch biopsies (4 mm diameter) were obtained from frozen skin areas using ethyl chloride for local freezing anesthesia. The still frozen specimens were immediately placed in and kept in liquid nitrogen.

Preparation of specimens

The frozen biopsies were placed in preheated buffer and kept at 60–65°C in a water bath for 10 min. After heating, the epidermis could be peeled off easily. Immediately after separation the epidermis was lyophilized to obtain dry weight. For extraction of cAMP, 100 μ l 0.1 N HCl was added and the sample homogenized for 3 min. The homogenate was transferred to fresh test tubes and combined with 2×100 μ l 0.1 N HCl used for washing the homogenizer. The resulting 300 μ l was boiled for 5 min, followed by centrifugation at 3500 g for 10 min. For purification the supernatant was lyophilized and redissolved in 300 μ l distilled water, followed by addition of 100 μ l 0.3 N ZnSO₄ and 100 μ l 0.3 N Ba(OH)₂ adjusted to pH 7.5. This mixture was incubated for 10 min in an ice bath and then centrifuged at 3500 g for 20 min. The supernatant was lyophilized and, after addition of 125 μ l assay buffer, 2×50 μ l was used for the analysis of cAMP.

Cyclic AMP assay

The RCA assay kit for cyclic AMP analysis is based on a competitive protein binding method. The assay was performed at 4°C. 50 μ l standard or sample, 50 μ l labelled cAMP and 100 μ l binding protein were added to test tubes and mixed. Standards and samples were always run in duplicate and a complete standard curve was included in each assay.

The test tubes covered with parafilm were then incubated for 120 min at 4°C. At the end of the incubation period, 100 μ l of the charcoal suspension was added, the tubes were mixed for 10 sec and then immediately centrifuged for 5 min at 3500 g at room temperature. Two hundred μ l of the supernatant was transferred to counting vials and 10 ml scintillation fluid added. The samples were subsequently counted at least twice for 5 min. Standard curves were plotted on semilogarithmic paper with percentage binding as a function of added cAMP. A full report on cAMP determination in heat-separated epidermal tissue has been published previously (11).

Table 1. Cyclic AMP levels in psoriatic epidermal tissue

Patient	Age	Uninvolved (pmol cAMP per mg dry weight)	Involved (pmol cAMP per mg dry weight)
1	27	4.18	3.15
2	24	6.10	1.80
3	30	5.67	2.37
4	35	4.70	2.92
5	40	6.93	3.70
6	46	6.26	2.11
7	57	2.14	1.12
8	40	15.2	7.05
9	71	18.5	11.9
10	30	10.0	7.1
Mean		7.97	4.32
S.E.M.		1.63	1.06

Student's *t*-test for paired data: $P < 0.001$.

RESULTS

In all patients a definite lower level of cAMP was detected in the psoriatic lesion when compared with normal-appearing skin from the same patient.

The results are given in Table 1. The total number of patients originally included in the study was 16 but biopsies from 6 patients were discarded because of incomplete epidermal separation with clusters of epidermal cells adhering to the dermis. Correct separation was monitored by histological examination of serial sections of the dermal part not used for cAMP measurements. As previously observed (11) dermal tissue was never found to adhere to the epidermis.

DISCUSSION

In the late 'sixties Voorhees and co-workers put forward as a working hypothesis that imbalanced cyclic nucleotides might be implicated as an underlying pathogenetic mechanism for the incomplete differentiation and abnormal proliferation in psoriatic epidermal tissue. The hypothesis was based on their original data demonstrating a decreased level of cAMP in tissue from psoriatic lesions (10).

During subsequent years several studies lending support to the theory of a defective epidermal cAMP cascade in psoriasis followed (1, 2, 7). Furthermore, clinical trials with topical application of drugs which *in vitro* had been able to increase epidermal cAMP were demonstrated to alleviate psoriasis moderately (3, 9). However, studies per-

formed by other laboratories (4, 5) and latest by Voorhees' own group (6) were unable to confirm the original findings. On the contrary, high levels of cAMP were detected in psoriatic lesions. The results over the years regarding the various levels of cAMP found in psoriasis are given in Table II.

The contradictory data obtained by the different laboratories prompted a debate on the validity of the proposed working hypothesis (4) with arguments focusing on the different techniques applied. Recently, Peters & While demonstrated a biphasic alteration in cAMP level in a model system of epidermal hyperplasia and hyperkeratinization induced by application of *n*-hexadecane to shaved rat skin. An initial decrease in cAMP associated with increased glycogenesis and DNA synthesis was followed by a prolonged supranormal level of cAMP (8). In all the earlier reported cAMP studies on psoriasis caution was taken to use only tissue specimens which had not been topically treated for at least one week, but no registration or concern about the 'age' of the investigated lesions is mentioned. Thus in these earlier reports it is uncertain whether the biopsied psoriatic lesion was a newly developed, a stable or a regressing one.

Since heat is inefficient in separating the epidermis from dermis in fully developed psoriatic lesions, we have only used newly appeared guttate elements for cAMP measurements and in these developing lesions we have demonstrated a definitely decreased level of cAMP in all patients investigated. These results are in agreement with those originally obtained by Voorhees et al. but in conflict with those reported by Halprin et al. and Härkönen et al. A theoretically conceivable ischemic effect

Table II. Cyclic AMP levels in uninvolved and involved psoriatic epidermis in the literature

Reference	Uninvolved psoriatic epidermis (pmol per mg wet weight)	Involved psoriatic epidermis (pmol per mg wet weight)
Voorhees et al. (10)	1.4	0.9
Härkönen et al. (5)	1.62	2.0
Halprin et al. (4)	0.91 ^a	1.16 ^a
Marcelo et al. (6)	0.321	0.321
Present data	2.66 ^a	1.44 ^a

^a The data have been converted from the original dry weight basis to a wet weight basis using the ratio of dry weight to wet weight of 1:3.

during heat separation cannot be completely ruled out, as previously discussed by us (11).

However, if the changes in psoriasis can be considered as an analogue to the events taking place in the hyperplastic rat skin, as reported by Peters & White, any earlier controversy between the hitherto obtained data may now be ignored.

Sequential changes in a biphasic manner with low cAMP in developing guttate lesions and supranormal levels of cAMP in stable lesions may account for the conflicting data and these may merely reflect the various levels of cAMP in psoriatic biopsy specimens at different developmental stages.

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Ultrastructural Study of Mechanobullous Desquamative Gingivitis: A Case Report

S. Kossard, R. K. Lofgren and
R. S. Rogers, III

*Department of Dermatology, Mayo Clinic,
Rochester, Minnesota, USA*

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Abstract. A 67-year-old female with localized bullous desquamative gingivitis is described. Ultrastructural studies of the perilesional area showed changes similar to those seen in cicatricial pemphigoid and epidermolysis bullosa acquisita.

Key words: Desquamative gingivitis; Cicatricial pemphigoid; Epidermolysis bullosa acquisita

Chronic desquamative gingivitis is a descriptive term for a clinical finding which may result from such diverse conditions as atypical gingivostomatitis, lichen planus, pemphigus, bullous pemphigoid or cicatricial pemphigoid (7, 8). Recently, patients with a chronic form of desquamative gingivitis and a marked sensitivity to trauma and absence of skin or extra-oral involvement have been felt to possibly represent a localized form of cicatricial pemphigoid or acquired epidermolysis bullosa (4).

We wish to describe the ultrastructural findings in a patient who represents this subtype.

CASE REPORT

The patient is a 67-year-old white female who presented with a 2½ year history of sore and bleeding gums. The condition was aggravated by mastication or toothbrushing. Blisters up to 1 cm in diameter, localized to the gingiva, have developed occasionally but no history of lesions involving other mucous membranes was noted.

A biopsy done elsewhere showed separation of the epidermis from the dermis. She had been treated for 1½ years with Prednisone 40 mg per day, which was reduced to 5 mg per day prior to being seen at the Mayo Clinic.

On physical examination, there were two ulcers, each 2 mm in diameter, on the lower inner gingiva surrounded by erythema. Probing of the ulcer edge with a blunt probe resulted in the epidermis being easily lifted from the dermis, with subsequent bleeding. The rest of the physical examination was normal.

Perilesional biopsy of the gingiva showed multiple focal subepidermal bullae associated with a chronic inflammatory infiltrate consisting of plasma cells, lymphocytes and