Rapid Effect of Treatment of Psoriatic Erythrocytes with the Synthetic Retinoid Acitretin to Increase 8-Azido Cyclic AMP Binding to the RI Regulatory Subunit

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We have recently demonstrated a deficiency in the cyclic adenosine monophosphate (cAMP)-dependent protein kinases (PKA), the intracellular mediator of AMP, in psoriasis. This enzyme defect is expressed in fibroblasts and in red blood cells isolated from psoriatic patients. In these cells, the abnormality noted in cAMP binding to PKA correlates well with the severity of the disease and is corrected by long-term treatment with etretinate. In this study, we determined the effect of oral administration of acitretin in four psoriatic patients on the altered cAMP binding observed with the RI regulatory subunit of PKA in erythrocytes prepared from these patients. Acitretin (30 mg/day) induced a rapid (within 1 h) increase in the ability of the RI regulatory subunit of erythrocytes to bind the 8-azido[^{32}P]cAMP photoaffinity analogue of cAMP. The maximal plateau for this effect of acitretin was observed within 24 h of treatment and preceded the clinical improvement of the disease. The effect of acitretin was dose-dependent, with the maximal response observed at 40 mg acitretin/d. In addition, the rapid exposure (15 min) of erythrocytes isolated from untreated patients exhibiting severe psoriasis to acitretin also promoted an increase in binding of 8-azido[^{32}P]cAMP to the RI cAMP binding protein. Retinoic acid and 13-*cis*-retinoic acid were as efficient as acitretin in inducing the increase in binding of 8-azido[^{32}P]cAMP to the RI regulatory subunit, whereas arotinoid was without effect. These results suggest that acitretin may act to modify PKA (the RI regulatory subunit) at the post-transcriptional level, and this may reflect, in part, on the mechanism of action of this synthetic retinoid. Further, monitoring this biochemical event may be helpful in determining the choice of retinoid therapy and in the management of its pharmacology. *J Invest Dermatol 100:77–81, 1993*

The etiology of psoriasis remains unclear; however, over the past two decades, a number of pathomechanisms have been advanced. Among them there is considerable evidence to suggest a role for abnormal cyclic adenosine monophosphate (cAMP) metabolism in the manifestation of the disease [1–4]. Recently, we have demonstrated a deficiency in the cAMP-dependent protein kinases (PKA), the intracellular mediator of cAMP, in psoriasis [5]. This enzyme defect is expressed in fibroblasts and in red blood cells isolated from psoriatic patients. In these cells, the abnormality noted in cAMP binding to PKA correlates well with the severity of the disease as well as its clinical evolution [6]. Of interest is the observation that retinoic acid treatment of psoriatic fibroblasts in culture induces an increase in cAMP-dependent PKA activity to levels found in normal human fibroblasts [7].

Retinoic acid derivatives have been used with some success in the therapeutic treatment of psoriasis. Etretinate and more recently its acid metabolite, acitretin, are the two synthetic retinoids that appear to be the most effective clinically in the treatment of psoriasis (see for review [8]). We previously showed in a long-term study that administration of oral etretinate in psoriatic patients resulted in a correction in the defective binding of 8-azido-[^{32}P]cAMP to the RI regulatory subunits of PKA observed in studies with erythrocytes isolated from these patients [5]. Therefore it was of interest to further characterize this effect of retinoid derivatives on the abnormal cAMP binding found in psoriatic erythrocytes. In this study, we demonstrate that oral administration of acitretin to psoriatic patients induces a very rapid (within 1 h) increase in the capability of the RI regulatory subunit of erythrocytes to bind the 8-azido-[^{32}P]cAMP analogue of cAMP in a dose-dependent manner. In addition, the rapid exposure of erythrocytes isolated from untreated patients exhibiting severe psoriasis to acitretin, retinoic acid, and 13-*cis*-retinoic acid also promotes an increase in binding of this cAMP analogue to the RI cAMP binding protein.

MATERIALS AND METHODS

Subjects Thirteen patients with psoriasis vulgaris were studied. The severity of the disease was evaluated by the Psoriatic Area and

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Abbreviations:
cAMP: cyclic adenosine monophosphate
EDTA: ethylenediaminetetraacetic acid
PKA: protein kinases
$NaDodSO_4$: sodium dodecyl sulfate

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Table I. Clinical and Biochemical Characteristics of the Patients Studied

Patient Number	Gender[a]/ Age (years)	PASI Score	8-Azido-[^{32}P] cAMP Bound[b] (fmol/μg membrane protein)
1	M/31	19.0	510
2	M/40	22.8	408
3	M/54	12.8	582
4	M/59	30.9	385
5	M/80	13.8	550
6	M/55	42.4	210
7	F/20	25.3	532
8	F/64	20.0	478
9	F/57	16.5	490
10	M/52	5.7	752
11	M/52	7.2	615
12	M/22	10.2	689
13	M/45	24.3	380

[a] M, male; F, female.

[b] Photoaffinity labeling with 8-azido-[^{32}P]cAMP of the RI regulatory subunit in erythrocyte membranes of the patients analyzed, as described in *Materials and Methods*.

Severity Index (PASI score), as previously defined [5]. These patients had received no specific treatment; no systemic therapy, such as corticosteroids, antimitotic drugs, or retinoids, for at least 1 year prior to this study; no non-steroidal anti-inflammatory drugs for at least 2 weeks prior to the assay; and no local corticosteroids, ultraviolet light, or oral or topical psoralene for at least 3 months prior to the assay. Subjects took part in this study only after they had given informed consent to the protocol approved by the Ethical Committee of Hospital Cochin Port Royal.

Oral Acitretin Treatment Acitretin was administered as a single dose, as indicated, per day following a continental breakfast.

***In Vitro* Retinoid Treatment** Thirty milliliters of blood were drawn in heparinized tubes. The blood sample was centrifuged at 1000 × *g* for 10 min at room temperature. The upper ring of white cells was carefully removed, and the erythrocyte pellet was resuspended with one-third its volume of plasma from the subject. The resuspended erythrocytes were incubated for the indicated time in the presence and absence of 1 or 0.1 μM of acitretin, retinoic acid, 13-*cis*-retinoic acid, or arotinoid, as indicated at 4°C, and treated erythrocytes were recovered from the incubation mixture by centrifugation at 1000 × *g* for 10 min at 4°C. All further steps were carried out at 4°C.

Preparation of Erythrocyte Membranes Erythrocyte membranes were prepared according to a modification of the procedure of Dodge *et al* [9]. A 10-ml sample of heparinized blood was obtained and immediately centrifugated for 30 min at 1000 × *g* at 4°C. The erythrocyte pellet obtained was suspended in 10 ml of 0.172 M Tris-HCl (pH 7.6) and washed three times with 10 ml of this buffer. The washed erythrocytes then were lysed by suspension in six volumes of hypotonic 0.011 M Tris-HCl (pH 7.6), and erythrocyte membranes were isolated by centrifugation at 2000 × *g* for 40 min. The membrane pellet was washed three times with 0.011 M Tris-HCl (pH 7.6) solubilized by resuspension in 1% NP-40 detergent for 5 min at 4°C and then stored frozen at −80°C until used for assay.

Photoaffinity Labeling with 8-Azido[^{32}P]cAMP RI regulatory subunits were photoaffinity labeled as described by Walter *et al* [10] in a reaction mixture (80 μl) containing 10 mM 2[N-morpholino]ethane sulfonic acid (MES), pH 6.2/10 mM $MgCl_2$/1.0 mM 8-azido-[^{32}P]cAMP, along with 100 μg of crude red cell membrane protein. Where indicated, 100 μM cAMP was included to block 8-azido-[^{32}P]cAMP binding to determine non-specific labeling. Mixtures were incubated for 60 min in the dark at 4°C and then irradiated for 10 min with an ultraviolet lamp to allow irreversible photoaffinity binding of 8-azido-[^{32}P]cAMP to the RI subunit. The irradiated samples were pipetted into 20 ml of stop solution [9% sodium dodecyl sulfate (SDS) 15% (v/v) glycerol/6 mM ethylenediaminetetraacetic acid (EDTA)/250 mM Tris-HCl, pH 8] and heated at 100°C for 20 seconds. Then, 2 μl of 2-mercaptoethanol and 5 μl of 0.1% bromophenol blue in 50% (v/v) glycerol were added, and the samples were electrophoresed in 10% polyacrylamide slab gels containing SDS. The gels were dried and autoradiographed at −80°C using Cronex 4 Dupont medical X-ray film. The autoradiographs were quantitated by scanning with a microdensitometer. One hundred micrograms of protein labeled with 8-azido-[^{32}P]cAMP were applied to each gel lane to allow comparison among the different lanes and autoradiographs. When care was taken not to overexpose the X-ray film, the peak heights obtained by scanning were proportional to the total radioactivity of the corresponding peaks estimated by scintillation counting, as described by Walter *et al* [10]. Levels of RI regulatory subunit were calculated by integrating the areas under the curves and subtracting non-specific labeling noted in the presence of 100 μM cAMP. Determinations for each subject were carried out in duplicate, and duplicates were found to agree within ±10%.

Protein Determination Protein concentrations are determined by fluorometric assay [11], with bovine serum albumin as the standard.

Table II. Time-Course of the Effect of Oral Administration of Acitretin in Four Psoriatic Patients on the Binding of 8-Azido-[^{32}P]cAMP to the RI Subunit Present in Erythrocytes

Patient		Time-Course				
		1 Hour	1 Day	8 Days	15 Days	1 Month
1	8-Azido-[^{32}P]cAMP binding[a]	—	1090	954	1818	1009
	PASI score	19.0	17.0	12.9	12.2	9.0
2	8-Azido-[^{32}P]cAMP binding[a]	160	368	362	417	316
	PASI score	22.8	22.0	18.0	15.0	6.5
3	8-Azido-[^{32}P]cAMP binding[a]	349	737	419	1357	1130
	PASI score	12.8	12.0	9.0	5.0	3.0
13	8-Azido-[^{32}P]cAMP binding[a]	268	—	152	—	350
	PASI score	24.3	—	19.0	—	5.0

[a] Percent of the value determined at the beginning of the treatment (see Table I).

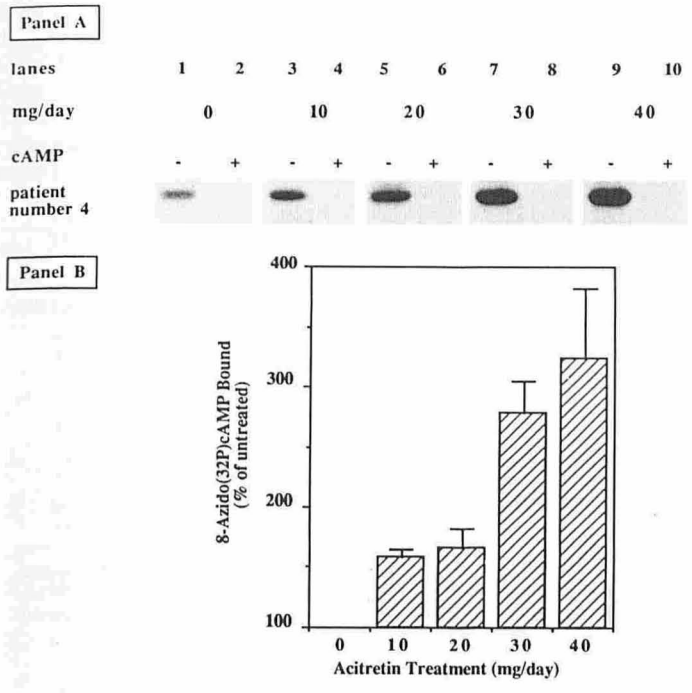

Figure 1. Effect of increasing doses of orally administered acitretin to a psoriatic subject on the binding of the cAMP analogue 8-azido-[^{32}P]cAMP to the RI regulatory subunit present in erythrocyte membranes isolated from the patient. *A*) Autoradiographs showing the photoactivated incorporation of 8-azido-[^{32}P]cAMP into the RI regulatory subunit analyzed by SDS polyacrylamide gel electrophoresis in erythrocytes prepared from patient 4. *B*) Quantitation of the amount of 8-azido-[^{32}P]cAMP incorporated into the RI regulatory subunit was carried out as described in *Materials and Methods.* Results are expressed as mean ± SD of the percent of untreated control values in three psoriatic patients (patients 4, 5, and 13). The psoriatic patients first were administered 10 mg of acitretin every 24 h for 7 d, and erythrocytes then were prepared from blood drawn on the eighth day. This was immediately followed by a similar regimen of treatment for 7 d with 20 mg of acitretin, followed by 30 mg of acitretin for 7 d, and finally 7 d of treatment with 40 mg of acitretin.

RESULTS

As shown in Table I, 13 patients with severe psoriasis as evaluated by the PASI score were used as subjects in this work. Erythrocyte membranes prepared from erythrocytes obtained from these patients all exhibited decreased binding of 8-azido-[^{32}P]cAMP to the RI regulatory subunit compared with normal subjects (normal values of 8-azido-[^{32}P]cAMP photoaffinity labeling of the RI regulatory subunit are 930 ± 35 fmol 8-azido-[^{32}P]cAMP bound/100 μg erythrocyte membrane protein [6]).

The time-course of the effect of oral acitretin treatment on the binding of the photoaffinity cAMP analogue to the erythrocyte membrane RI regulatory subunit was studied in four of the psoriatic patients (Table II). Oral acitretin treatment (30 mg/d) produced a significant increase in the amount of specific binding of 8-azido-[^{32}P]cAMP to the RI regulatory subunit of PKA in erythrocyte membranes. Interestingly, this increase occurred very rapidly and was clearly observed within 1 h of acitretin treatment in three of the patients. The plateau of the effect of acitretin was obtained 24 h after the first ingestion of the retinoid and preceded the clinical improvement in the disease (within 8 d), as noted by the PASI score (Table II).

The capability of acitretin to induce normal binding of the cAMP analogue to the RI regulatory subunit in red blood cell membranes prepared from treated psoriatic patients was dose-dependent (Fig 1). In psoriatic patient 4, the maximal effect was observed with the oral administration of 40 mg of acitretin (Fig 1*A*). As shown in Fig 1*B*, increasing doses of acitretin treatment from 10 to 40 mg/d in three psoriatic patients (patients 4, 5, and 13) induced a progressive increase in 8-azido-[^{32}P]cAMP binding to the RI regulatory subunit to a maximum of 323 ± 59% (mean ± SD) of the value observed prior to the onset of treatment. The interruption of acitretin treatment for 8 d (after 2 weeks of acitretin treatment at 40 mg/d), in psoriatic patient 4 resulted in a reversal of the effect to again exhibit the decreased levels of 8-azido-[^{32}P]cAMP binding to the RI regulatory subunit (a decrease from 1059 to 153 fmol 8-azido-[^{32}P]cAMP bound/100 μg erythrocyte membrane protein). This level was characteristic of erythrocytes prepared from severely psoriatic patients prior to acitretin treatment; however, a short interruption in acitretin treatment (for 2 days) induced only a 40% decrease in the capability of the RI regulatory subunit to bind the cAMP analogue (preliminary data not shown).

This very rapid effect of orally administered acitretin on the regulatory subunit of PKA in red blood cells led us to determine whether acitretin might have a similar effect *in vitro* on isolated erythrocytes. As shown in Table III, acitretin treatment of isolated erythrocytes obtained from patients with severe psoriasis induced a significant increase in the 8-azido-[^{32}P]cAMP binding to the RI regulatory subunit. In contrast, no significant effect of acitretin on erythrocytes obtained from normal subject was observed on the 8-azido-[^{32}P]cAMP binding to the RI subunit in normal red blood cells. This *in vitro* effect of acitretin on psoriatic erythrocytes was dose-dependent, with a maximal increase in binding observed with 10^{-8}–10^{-7} M of acitretin (Fig 2). Retinoic acid and 13-*cis*-retinoic acid were as effective as acitretin in increasing the binding of 8-azido-[^{32}P]cAMP to the RI regulatory subunit, whereas arotinoid was ineffective (Table III). Again, no *in vitro* effect of these retinoids was observed on RI binding with erythrocytes from normal subjects.

DISCUSSION

Previous studies have established a close relationship between retinoic acid and cAMP to mediate a number of biologic responses, particularly cell growth and differentiation. Many cell types, including myeloid leukemia, teratocarcinoma, melanoma, and kidney cells, are responsive to this treatment (for review, see [12]). In psoriatic fibroblasts, sequential exposure to retinoic acid and then to 8-Br-cAMP induces a decrease in cell growth, whereas 8-Br-cAMP alone is only marginally effective in decreasing psoriatic fibroblast growth [13]. This treatment with retinoic acid may induce changes that alter the response to cAMP, and evidence in different cell types suggests that changes in PKA activity may be one of the targets of retinoic acid action [14–17]. Indeed, PKA appear to be largely, if not solely, responsible for carrying out the biologic effects of cAMP. There are two major forms of PKA (types I and II). These forms differ in the nature of their cAMP binding regulatory subunits (RI and RII). Human erythrocyte membranes contain RI but not RII [18,19].

We have demonstrated in psoriatic cells that there is a defect in the PKA expressed not only in fibroblasts, but also in red blood cells. The capacity of the regulatory subunits to bind cAMP and its analogue, 8-azido[^{32}P]cAMP, seems to be abnormal in erythrocytes isolated from psoriatic patients [6].

Retinoid treatment of psoriatic fibroblasts in culture induces an increase in cAMP-dependent PKA activity [7]. In addition, we have shown that systemic treatment of psoriatic patients with etretinate resulted in a reversion of the binding defect of the cAMP analogue 8-azido[^{32}P]cAMP to the PKA toward normal level [6].

Acitretin is the acid metabolite of etretinate. Both of these aromatic retinoids belong to the second-generation family of retinoids. Acitretin offers the clinical advantage of not being stored in adipose tissue. Clinical trials evaluating acitretin in the treatment of psoriasis parallel the investigation of etretinate [20]. *In vitro* experiments demonstrate that acitretin, like etretinate, can influence the chemotaxis of granulocytes, inhibit the proliferative response of lymphocytes to mitogens, and influence keratine synthesis in culture. In this

Table III. *In Vitro* Effect of Retinoids on the Level of 8-Azido[^{32}P]cAMP Specifically bound to the RI Regulatory Subunit of cAMP-Dependent Protein Kinase Present in the Membrane Fraction of Red Blood Cells[a]

Patient Number or Letter	Retinoid Concentration (μM)	8-Azido[^{32}P]cAMP Binding (% of Untreated Control)			
		+Retinoic Acid	+Acitretin	+13-*cis*-Retinoic Acid	+Arotinoid
Psoriatic patients					
5	1	140	152	149	110
8	1	120	139	130	100
6	1	—	153	—	—
9	1	—	150	—	—
10	0.1	154	151	150	109
11	0.1	130	148	134	96
12	0.1	155	161	154	105
Normal subjects					
A	1		103	—	—
B	1	100	110	107	98
C	1	—	109	—	—
D	1	100	103	101	97
E	0.1	100	100	102	100
F	0.1	100	101	102	96

[a] Erythrocytes were treated *in vitro* for 15 min with either 0.1 or 1 μM of retinoic acid, acitretin, 13-*cis*-retinoic acid, or arotinoid. Then, erythrocyte membranes were prepared, and photoaffinity labeling with 8-azido-[^{32}P]cAMP was carried out, as described in *Materials and Methods*. The binding of 8-azido-[^{32}P]cAMP to the RI regulatory subunit was analyzed by SDS polyacrylamide gel electrophoresis. The amount of [^{32}P] bound was quantitated by cutting the radioactive band from the gel and determining the amount of radioactivity present in the solubilized band with a scintillation counter. Results are expressed as percent of the control value listed in Table I.

study, we show that acitretin treatment, as well as etretinate treatment [6], can correct the binding defect of PKA in psoriatic patients.

Our previous study with long-term treatment with etretinate [6] did not allow us to determine whether the observed enhancement in photoaffinity labeling of RI by 8-azido[^{32}P]cAMP was directly related to the retinoid treatment or, less specifically, to an improvement in the disease state. In this study, we clearly demonstrate that retinoid treatment (i.e., the oral intake of acitretin) very rapidly (within 1 h) corrects the low binding noted in erythrocytes from untreated psoriatic patients. This effect of orally administered acitretin is dose dependent. These data suggest a direct and immediate effect of acitretin on the PKA or erythrocytes of the psoriatic patients. This is further suggested by preliminary data obtained in one psoriatic subject. Interruption of treatment with acitretin for an interval of time (8 d) that is greater than the elimination half-life of acitretin (50 h), is followed by the reappearance of the binding defect observed with the RI subunit of psoriatic erythrocytes. In contrast, a short interruption of acitretin treatment (<48 h) allows only a small decrease in binding of the cAMP analogue toward that noted with cells from untreated psoriatic patients.

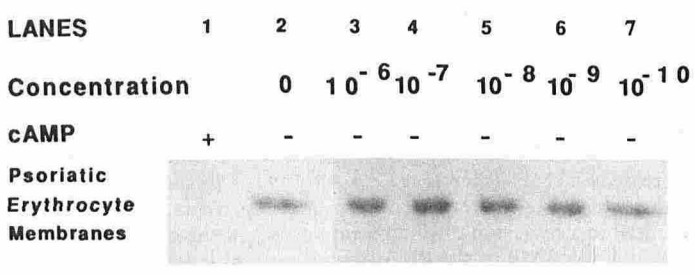

Figure 2. Effect of treatment of isolated psoriatic erythrocytes for 15 min with increasing concentrations of acitretin on the binding of 8-azido-[^{32}P]cAMP to the RI regulatory subunit. The amount of the 8-azido-[^{32}P]cAMP photoaffinity analogue bound to the RI regulatory subunit following treatment of the isolated erythrocytes with the indicated concentrations of acitretin for 15 min was determined by SDS polyacrylamide gel electrophoresis followed by autoradiography, as described in *Materials and Methods*.

The mechanisms responsible for the effects of retinoic acid and its synthetic derivatives have not been fully elucidated. An important insight has been provided by the discovery of three retinoic acid receptors (α, β, γ). The retinoic acid receptors are believed to function in a manner analogous to other members of the steroid/thyroid hormone receptor family with which they have a high degree of sequence similarity [21–25]. Retinoic acid receptor-γ is reported to be the type expressed in the skin [26]; however, the mechanism of action of retinoids to modulate cAMP-dependent PKA remains to be elucidated. Retinoid treatment of psoriatic cells may induce an elevation of PKA activity either by increasing PKA protein levels or by inducing a modification of the enzyme to influence cAMP binding and phosphotransferase activity. As shown in this study, the effect of orally administered acitretin to increase the binding of 8-azido-[^{32}P]cAMP to the RI cAMP binding regulatory subunit of PKA is very rapid. A similar response is observed with *in vitro* treatment of isolated psoriatic erythrocytes with acitretin. This *in vitro* effect is observed within minutes and occurs in anucleated cells. These data suggest that acitretin acts to modify the cAMP-dependent PKA at a post-transcriptional level.

In this regard, retinoylation (retinoic acid acylation) is a covalent modification of specific proteins that occurs in a variety of eukaryotic cell lines [27]. Recently, it was reported that both the RI and RII regulatory subunits of PKA appear to be retinoylated [28]. Studies are in progress to determine whether retinoylation of RI and RII occurs in psoriatic cells and whether this type of modification might alter the binding of 8-azido[^{32}P]cAMP to the regulatory subunits.

Previously, we showed that long-term treatment of psoriatic fibroblasts with retinoic acid and with other retinoids, such as 13-*cis*-retinoic acid and arotinoid, resulted in increased cAMP-dependent PKA activity [7]. In the present study, rapid (15 min) treatment of anucleated erythrocytes with arotinoid had no effect on the capability of the RI regulatory subunit to bind the cAMP analogue, whereas the other retinoids tested were effective. The discrepancy between these *in vitro* results with arotenoid might be explained by the different experimental conditions used. These results suggest a possible dual regulation of PKA with a rapid effect related to post-transcriptional modification of the enzyme and a more long-term effect related to altered gene expression.

The ease in obtaining erythrocytes from patients and the presence of this defect in cAMP-dependent PKA in erythrocytes obtained

from psoriatic patients present a useful cell population to determine the presence and the evolution of the disease. This *in vitro* effect of retinoids on PKA in psoriatic erythrocytes may reflect on the mechanism and efficacy for the *in vivo* therapeutic effects of retinoids. Thus, initial studies on the retinoid effect on RI in psoriatic erythrocytes may be helpful in determining the choice of therapy and the management of its pharmacology.

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REFERENCES

1. Voorhees JJ; Psoriasis as possible defect of adenylcyclase cyclic AMP cascade. Arch Dermatol 118:863–868, 1982
2. Voorhees JJ, Duell EA: Imbalanced cyclic AMP, cyclic GMP levels in psoriasis. Adv Cyclic Nucleotide Protein Phosphorylation Res 5:735–758, 1975
3. Marcelo CL, Voorhees JJ: Cyclic nucleotides, prostaglandins, and polyamines in psoriasis. Pharmacol Ther 9:297–310, 1979
4. Kumar R, Weiss VC, West DP, Chaiero LA: Altered erythrocyte membrane phosphorylation in psoriasis. Br J Dermatol 109:267–275, 1983
5. Evain-Brion D, Raynaud F, Plet A, Laurent P, Leduc C, Anderson WB: Deficiency of cyclic AMP dependent protein kinases in human psoriasis. Proc Natl Acad Sci USA 83:5272–5276, 1986
6. Raynaud F, Gerbaud P, Enjolras O, Gorin I, Donnadieu M, Anderson WB, Evain-Brion D: A cAMP binding abnormality in psoriasis. Lancet I:1153–1156, 1989
7. Raynaud F, Leduc C, Anderson WB, Evain-Brion D: Retinoid treatment of human psoriatic fibroblasts induces an increase in cyclic AMP dependent protein kinase activity. J Invest Dermatol 89:105–110, 1987
8. Gollnick HP, Orfanos CE: Clinical efficacity of etretinate and acitretin. In: Roenigk HH, Maibach HI (eds.). European Experience Psoriasis. Marcel Dekker, New York, 1991, pp 725–748
9. Dodge JR, Mitchell CD, Hanahan DJ: The preparation and chemical characteristics of hemoglobin-free ghosts of human erythrocytes. Arch Biochem Biophys 100:119–130, 1963
10. Walter V, Uni I, Liu AY, Greengard P: Identification, characterization and quantitative measurements of cyclic AMP receptor proteins in cytosol of various tissues using a photoaffinity ligand. J Biol Chem 252:6494–6500, 1977
11. Bohlen P, Stein S, Dairman W, Unfriend S: Fluorometric assay of proteins in the nanogram range. Arch Biochem Biophys 155:213–229, 1973
12. Evain-Brion D, Raynaud F, Tournier S, Plet A, Anderson WB: Retinoic acid and cellular signal transduction. Saurat J-H (ed.). Retinoids: 10 Years On. Karger, Basel, 1991, pp 46–55
13. Evain-Brion D, Raynaud F, Plet A, Laurent P, Leduc B: Effects of retinoids on cAMP-mediated events in human psoriatic fibroblasts. Growth and cAMP-dependent protein-kinases. Saurat J-H (ed.). Retinoids: New Trends in Research and Therapy. Karger, Basel, 1984, pp 189–193
14. Plet A, Evain D, Anderson WB: Effect of retinoic acid treatment of F9 embryonal carcinoma cells on the activity and distribution of cyclic AMP dependent protein kinases. J Biol Chem 257:889–893, 1982
15. Ludwig KW, Loewy B, Niles RM: Retinoic acid increases cAMP dependent protein kinase activity in murine melanoma cells. J Biol Chem 255:5999–6002, 1980
16. Elias L, Stewart T: Subcellular distribution of cyclic AMP dependent protein kinase during the chemically induced differentiation of HL-60 cells. Cancer Res 44:3075–3080, 1984
17. Rogelj S, Loewy B, Niles RM: The effect of retinoic acid on cyclic AMP binding protein in mouse melanoma cells. Eur J Biochem 139:351–357, 1984
18. Dreyfuss G, Schwartz KJ, Blout ER: Compartmentalization of cAMP dependent kinases in human erythrocytes. Proc Natl Acad Sci USA 75:5926–5930, 1978
19. Rubin CS: Characterization and comparison of membrane associated and cytosolic cAMP dependent protein kinases. J Biol Chem 254:12439–12449, 1979
20. Gollnick H, Bauer R, Brindley C, Orfanos CE, Plewig G, Wokalek H, Hoting E: Acitretin versus etretinate in psoriasis. J Am Acad Dermatol 19:458–469, 1988
21. Giguere V, Ong ES, Segui P, Evans RM: Identification of a receptor for the morphogen retinoic acid. Nature 330:624–629, 1987
22. Petkovitch M, Brand NJ, Kurst A, Chambon P: A human retinoic acid receptor which belongs to the family of nuclear receptors. Nature 330:144–150, 1987
23. Brand NJ, Petkovitch M, Krust A, Chambon P, de Thé H, Marchio A, Tiollais P, Dejean A: Identification of a second human retinoic acid receptor. Nature 332:850–853, 1988
24. Benbrook D, Lernhardt E, Pfahl M: A new retinoic acid receptor identified from a hepatocellular carcinoma. Nature 33:669–672, 1988
25. Krust A, Kastner Ph, Petkovitch M, Zelent A, Chambon P: A third human retinoic acid receptor h RAR-γ. Proc Natl Acad Sci USA 87:5310–5314, 1989
26. Zelent A, Krust A, Petkovitch M, Kastner P, Chambon P: Cloning of murine α and β retinoic acid receptors and a novel receptor γ predominantly expressed in skin. Nature 340:714–717, 1989
27. Takahashi N, Breitman TR: Retinoylation of HL-60 proteins. J Biol Chem 265:19158–19162, 1990
28. Takahashi N, Liapi C, Anderson WB, Breitman TR: Retinoylation of the cAMP-binding regulatory subunits of type I and type II cAMP-dependent protein kinases in HL60 cells. Arch Biochem Biophys 290:293–302, 1991