嘉南藥理學院教師專題研究計畫成果報告

計畫名稱：果酸對角質細胞增殖分化之影響

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摘要

果酸具有改善皮膚之效能，所以為一般大眾所喜愛，許多愛美人士更是趨之若鶩。但是果酸對皮膚的影響，卻不只有好的一面，也有其不好的一面。果酸最常見的副作用為皮膚紅腫、發紅、刺痛感、灼熱感、脫皮以及發癢等。果酸對皮膚所造成之潛在危險性—表皮的過度角化，引起皮膚對紫外線的敏感性，增加了光老化(photoaging)和皮膚癌之出現率，這更是目前在使用讓皮膚擁有短暫美麗的果酸產品時，更需要被注意的。為了瞭解果酸對皮膚角質細胞的影響，本研究針對果酸對角質細胞增殖及分化著手。本研究結果顯示在果酸的刺激下，角質細胞的增殖分化能力有顯著的改變。

關鍵字：果酸，角質細胞，增殖，分化

前言及本文

Epidemiological studies have shown that α-hydroxy acids (AHA) result in human skin hyperkeratosis [1]. Epidermal kerationcytes contain the β_2 -adrenergic adenylate cyclase system, which when activated causes an accumulation of intracellular cyclic AMP through stimulatory guanosine 5-triphosphate (GTP) binding proteins [2]. Adrenergic receptors are responsible for selective recognition and binding of catecholamines, affecting epidermal cell proliferation and differentiation [3]. β_2 -adrenergic receptor hyporesponsiveness has been observed in several skin diseases including psoriasis [4]. However, to the best of our knowledge, there is currently not reported for the effects of AHA on the expression of β_2 -adrenergic receptors in cultured keratinocytes. In the present study, we assessed the effect of AHA on the expression of β_2 -adrenergic receptors in cultured keratinocytes using a ^{125}I-iodocyanopindolol (^{125} ICYP) binding assay to examine receptor density.

The keratinocytes were obtained from the adult foreskin from a routine circumcision and

cultured in keratinocyte-SFM medium (Gibco, Grand Island, N. Y., USA) which contains 25 μg/ml bovine pituitary extract (BPE) and 5 ng/ml recombinant epidermal growth factor (rEGF). Keratinocytes at the third passage were then grown in keratinocyte-SFM medium without BPE and rEGF for at least 24 h before being exposed to various concentrations of AHA. Cells were gently washed twice with PBS and then solutions containing 0 M, 10^{-5} M, 10^{-6} M and 10^{-7} M AHA were added, respectively. After 24 h incubation, the treated cells were harvested and followed by ultracentrifugation for β_2 -adrenergic receptor binding assay. A modification of the β_2 -adrenergic receptor assay developed by Steinkraus et al. [5] was applied to bind ^{125}ICYP to the kertinocyte membrane. The β_2 -adrenergic receptor density (Bmax) and dissociation constant (Kd) for ^{125}ICYP binding was determined from the saturation curves of specific binding analyzed by the Scatchard method [6]. Protein was measured according to the method of Lowry et al. [7] using bovine serum albumin as the standard.

The results of our study are summerized in table 1. The β_2-adrenergic receptor density (Bmax) of normal control keratinocytes was 95.49 ±4.26 fmol/mg membrane protein, and the value in AHA-incubated keratinocytes was 72.41 ± 5.23, 60.34 ± 4.33 and 46.76 ± 5.02 fmol/mg membrane protein after incubation with 10^{-7} M, 10^{-6} M and 10^{-5} M AHA, respectively. This difference reached statistical significance (unpaired t-test, $p<0.05$). The Kd values were not statistically significant ($p>0.05$).

Epidermal keratinocytes possess a series of cell surface receptors for catecholamines [8], histamine [9], adrenosine [10, 11], and prostaglandins E_1 and E_2 [12] which are coupled to adenylate cyclase. while species differences do occur in the magnitude of response and order of sensitivity to a variety of agonists, Wilkinson and Orenberg [3] have shown that the adenylate cyclase of human keratinocytes is most sensitive to agents that stimulate the adrenergic receptor. That adrenergic receptor may be important with regard to regulation of epidermal growth is suggested by the fact that several laboratories have independently reported that involved psoriatic epidermis in vitro has a decreased response to catecholamines [4]. Also, in tissue slices of epidermis that had been treated with hexadecane to produce hyperplasia, the increased proliferation, amino acid incorporation, and glycolysis were associated with loss of responsiveness to β-adrenergic agonists [13]. These studies suggested that these was the close relation between epidermal hyperproliferation and decreased β-adrenergic responsiveness.

Voorhees and Duell suggested in 1971 that the β-adrenergic-adenylate cyclase-cAMP system of epidermal tissue may be crucial for its proliferative and differentiative homeostasis and that the responsiveness of this system may be decreased in psoriasis [14]. The finding of decreased β- adrenergic responsiveness in psoriasis was experimentally supported by several investigators [4,15]. It was further established from clinical experiences that therapy with β-adrenergic blocking drugs may lead to severe exacerbation of psoriatic lesions [16,17]. It has suggested that this side effect may reflect an interaction of β-adrenergic blocking drugs with epidermal β -adrenergic receptors. Moreover, Iizuka et al. [18] have reported that the decreased β-adrenergic

adenylate cyclase response in psoriatic epidermis is likely to be due to defective β-adrenergic receptors, or defective receptor coupling with the GTP-binding protein (G-protein). We therefore believe that decreased β-adrenergic response play a important role in epidermal hyperproliferation and hyperkeratosis.

The β-adrenoceptor-mediated function is attenuated by persistent exposure to Catecholamine [19]. The process has been referred to as down-regulation and has been correlated with the loss of β-adrenergic receptor [20]. In this study, we predict that the potential mechanism of the AHA-induced decrease in β-adrenoceptor-density may be related with the change of catecholamine level.

Our study showed that AHA incubation resulted in epidermal β-adrenergic receptor density decreased. Thus, β-adrenergic adenylate cyclase response reduced in AHA-incubated epidermis. We suggested that AHA induced epidermal hyperproliferation and hyperkeratosis is due to defective β-adrenergic receptors.

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Table 1. AHA affects the β2- adrenergic receptor density and affinity in cultured keratinocytes.

Group	Bmax (fmol/ mg)	Kd (pM)
Control	95.49±4.26	48.48±4.33
Dose of AHA		
10^{-7} (M)	72.41±5.23*	47.03±3.22
10^{-6} (M)	60.34±4.33*	50.67±4.60
10^{-5} (M)	46.76±5.02*	49.28±5.71

Bmax : β2-adrenergic receptor density. Kd : Concentration of ^{125}ICYP for 50% occupation of β-adrenergic receptor.

* $p<0.05$, unpaired t-test.