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Adapalene suppresses sebum accumulation *via* the inhibition of triacylglycerol biosynthesis and perilipin expression in differentiated hamster sebocytes *in vitro*

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ABSTRACT

Background: Acne is a chronic inflammatory disease in sebaceous glands and pilosebaceous units where excess sebum production and follicular hyperkeratinization are observed. Adapalene, which exerts comedolytic and anti-inflammatory effects, is used for the topical treatment of mild to moderate acne.
Objective: We examined the effect of adapalene on sebum production and accumulation in sebaceous gland cells (sebocytes).
Methods: The regulation of sebum production was examined by oil red O and nile red staining and the measurement of triacylglycerols (TGs) in differentiated hamster sebocytes. The gene expression and production of diacylglycerol acyltransferase-1 (DGAT-1) and perilipin 1 (PLIN1) were analyzed using real-time PCR and Western blotting, respectively.
Results: Adapalene suppressed sebum accumulation as lipid droplets in spontaneously and insulin-differentiated hamster sebocytes. The TG production, and the gene expression and production of DGAT-1, a rate-limiting enzyme of TG biosynthesis, were dose-dependently inhibited by adapalene in insulin-, 5α-dihydrotestosterone- or a peroxisome proliferator activating receptor γ agonist, troglitazone-differentiated hamster sebocytes. In addition, the inhibition of TG production by adapalene interfered with antagonists against nuclear retinoic acid and retinoid X receptors (CD2665 and UVI3006, respectively) in the differentiated sebocytes. Furthermore, the production of PLIN1, a lipid storage droplet protein, was transcriptionally inhibited by adapalene in the differentiated sebocytes.
Conclusions: These results suggest that adapalene exerts an inhibitory action for sebum accumulation due to the suppression of TG and PLIN1 production in differentiated hamster sebocytes. Furthermore, these findings may contribute to a novel understanding of the molecular mechanisms of adapalene for acne treatment and prevention.

1. Introduction

The pathogenesis of acne, a common inflammatory skin disease [1,2], is characterized by: (i) excess sebum production in sebaceous glands; (ii) the formation of microcomedones, which is closely associated with the hyperkeratinization of the follicular wall and infundibulum; and (iii) the hyperproliferation of *Propionibacterium acnes* (*P. acnes*), and (iv) the induction of inflammatory reactions such as the acceleration of cytokine production and the biosynthesis of arachidonic acid metabolites in keratinocytes, sebocytes, and invaded inflammatory cells [3,4]. The aggravation and duration of the inflammation is likely to result in a severely disfiguring and permanent sequel, acne scar that causes a psychological and social impact in the patient's quality of life [5–8]. Regarding the disorder of sebaceous lipogenesis, androgens such as testosterone and 5α-dihydrotestosterone (5α-DHT) have been reported to participate in excess sebum production and the enlargement of sebaceous glands in acne lesions [4]. In addition, insulin, insulin-like growth factor 1, prostaglandins (PGs), and peroxisome proliferators activating receptors (PPARs) have all been reported to contribute to the development and aggravation of acne [9–13].

Retinoic acids such as tretinoin (all-*trans* retinoic acid; atRA) and isotretinoin (13-*cis* retinoic acid; 13-*cis*RA) have been topically and/or systemically used for acne therapy [14,15]. They have been reported to have a comedolytic action due to the inhibition of follicular epithelium hyperkeratinization, and thereby the formation and number of microcomedones decrease in acne lesions [15].

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In addition, retinoids have been reported to exhibit an anti-inflammatory effect by inhibiting the production of proinflammatory cytokines and other mediators in skin disorders including acne [15,16]. Furthermore, both atRA and 13-*cis*RA have been reported to decrease sebum production and sebaceous gland enlargement by inhibiting lipogenesis and cell proliferation in humans, rats, and hamsters [17–20]. However, the use of retinoids in acne therapy has been limited in acceptance because of adverse effects such as skin irritation and scaling, and teratogenicity [14].

Adapalene, a synthetic naphthoic acid derivative, is a topical anti-acne agent with the equivalent clinical effects of tretinoin and isotretinoin and with a better tolerability than retinoids [21,22]. Adapalene has been reported to be topically effective for acne patients under non-inflammatory and inflammatory conditions due to the inhibition of comedone formation and cutaneous inflammatory reactions [16,21,22]. Since retinoids exert their biological activities *via* specific nuclear retinoic acid (RAR) and retinoid X (RXR) receptors [23], adapalene has been reported to bind selectively to RAR subtypes β and γ [21]. The adapalene-RAR complex sequentially binds to RXR, and then the adapalene/RAR/RXR interacts with the promoter region of target genes for the transcriptional regulation [21]. Although acne is characteristic of sebaceous disorders [1–4] and both atRA and 13-*cis*RA exhibit anti-lipogenetic actions in differentiated sebocytes [17–20], it is not fully understood whether adapalene directly suppresses sebum production in sebaceous glands or not.

In the present study, we demonstrated that adapalene inhibited the production and intracellular accumulation of sebum in insulin-, 5α-DHT-, and PPARγ agonist-differentiated hamster sebocytes in a RAR/RXR pathway-dependent manner. Furthermore, the adapalene-mediated inhibition of sebum production and accumulation are closely associated with the transcriptional suppression of diacylglycerol acyltransferase 1 (DGAT-1), a rate-limiting enzyme of triacylglycerol (TG) synthesis, and perilipin 1 (PLIN1), a lipid-droplet surface protein, respectively.

2. Materials and methods

2.1. Cell culture and treatment

Hamster sebocytes (2.4×10^4 cells/cm^2) [24] were plated onto 96-well multiplates, 35-mm or 100-mm diameter culture dishes (Becton Dickinson, Tokyo, Japan) and then cultured for 24 h in DMEM/F12 (Invitrogen, Carlsbad, CA) supplemented with 6% heat-denatured fetal bovine serum (JRH Bioscience, Tokyo, Japan), 2% human serum (ICN Biochemicals, Costa Mesa, CA), 0.68 mM L-glutamine (Invitrogen), and recombinant human epidermal growth factor (10 nM) (Progen Biotechnik GmbH, Heidelberg, Germany) to achieve complete cell adhesion as previously described [10,19]. For sebocyte differentiation, the hamster sebocytes were treated every two days for up to 10 days with insulin (10 nM), 5α-DHT (10 μM) (Sigma Chemical, St. Louis, MO) or troglitazone (10 μM) (kindly provided from Sankyo Co, Tokyo, Japan), under which intracellular lipid droplets were formed [25]. In this series of experiments, hamster sebocytes were used as far as the 3rd passage level.

2.2. Oil red O staining

After treating the sebocytes with insulin, adapalene and/or 13-*cis*RA, the cells were washed once with Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline [PBS(−)] and fixed with 4% paraformaldehyde (Wako Pure Chemicals, Osaka, Japan) diluted with PBS(−) for 1 h at room temperature. The cells were washed with distilled H_2O and then stained with 0.3% oil-red O (Sigma Chemical) in isopropanol:distilled H_2O (3:2, vol:vol) at 37 °C for 15 min. The stained cells were washed with distilled H_2O, and then viewed with a light microscope furnished with a digital camera (Olympus Optical Co, Tokyo, Japan).

2.3. TG measurement

Hamster sebocytes (1.8×10^5 cells) in the 35-mm diameter culture dish were treated every two days for up to 10 days with adapalene (6-[3-(1-adamantyl)-4-methoxyphenyl]-2-naphthoic acid) (1–100 nM) (kindly provided from Galderma, La Défense cedex, France), 13-*cis*RA (100 or 1000 nM) (Sigma Chemical), a selective RARβ/γ antagonist, CD2665 (4-[6-[(2-Methoxyethoxy)-methoxy]-7-tricyclo[3.3.1.13,7]dec-1-yl-2-naphthalenyl]benzoic acid) (1–100 μM), and/or a RXR antagonist, UVI3006 (3-[4-Hydroxy-3-[5,6,7,8- tetrahydro-5,5,8,8-tetramethyl-3-(pentyloxy)-2-naphthalenyl]phenyl]-2-propenoic acid) (0.01–1 μM) (Tocris Bioscience, Bristol, UK) in the presence or absence of insulin (10 nM), 5α-DHT (10 μM) or troglitazone (10 μM) in DMEM/F12 supplemented with heat-denatured fetal bovine serum, human serum, and L-glutamine. The harvested cells were subjected to TG quantification using Liquitech TG-II (Roche Diagnostics, Tokyo, Japan) as previously described [25]. The amounts of intracellular TG were calculated using an authentic trioleinate-standard solution (0.6 mg/ml). Intracellular DNA content was measured using salmon sperm DNA (6.25–100 mg/ml) and 3,5-diaminobenzoic acid dihydrochloride (Sigma Chemical).

2.4. Analysis of sebum production by nile red staining

Hamster sebocytes in 96-well multiplates were treated every two days for up to 10 days with insulin, 5α-DHT, and/or adapalene in the presence or absence of CD2665 or UVI3003. The cells were washed once with PBS(−) and fixed with 4% paraformaldehyde diluted with PBS(−) for 1 h at room temperature, and then stained with nile red (1 μg/ml) (Sigma Chemical) at 37 °C for 15 min. The fluorescent intensity of the nile red-stained cells was measured by a microplate reader, Infinite F200 (TECAN, Kanagawa, Japan), at 485 nm (excitation) and 565 nm (emission). The relative production of sebum was expressed as the mean value of the control as 100%.

2.5. Real-time PCR

For the quantification of DGAT-1 and PLIN1 mRNA, total RNA was isolated from cells using ISOGEN (Nippon Gene, Toyama, Japan) and then the aliquot of RNA (500 ng) was subjected to reverse transcriptase reaction for the synthesis of cDNA using a PrimeScript RT reagent Kit (Takara Bio, Shiga, Japan) according to the manufacturer's instructions. Aliquots (an equivalent of 2.5 ng of total RNA) of the transcript were subjected to real-time PCR using SYBR Premix Ex Taq II (Takara Bio) and following specific primers: human DGAT-1 (NM_012079); 5′-TCTACAAGCCCATGCTTCGAC-3′ (sense) and 5′-GGACGCTCACCAGGTACT-3′ (antisense), hamster PLIN1 (AB091681); 5′-ACCTTGCTGGATGGAGACC-3′ (sense) and 5′-CCAGGACCTTGTCTGAAGT-3′ (antisense), and hamster glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (X52123); 5′-CAGAACATCATCCCTGCAT-3′ (sense) and 5′-TAGGAACACG-GAAGGCCAT-3′ (antisense). The amplification cycle was performed at 94 °C for 5 s and 60 °C for 30 s using a Thermal Cycler Dice Real Time System TP-800 (Takara Bio). The obtained threshold cycle (CT) value for DGAT-1 and PLIN1 was normalized by that for GAPDH, and the relative expression level was expressed as the mean value of the control as 1.

2.6. Western blot analysis

The harvested cell lysate (50 μg protein) was subjected to Western blot analysis using 12.5% acrylamide gel. The membrane