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Inhibitory effect of imperatorin on insulin-like growth factor-1-induced sebum production in human sebocytes cultured in vitro

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ABSTRACT

Aims: Acne is a common skin disease that originates in the sebaceous gland. The pathogenesis of acne is very complex, involving the increase of sebum production and perifollicular inflammation. In this study, we screened the anti-lipogenic material and demonstrated its effect using cultured human sebocytes.
Main methods: Normal human sebocytes were cultured by explanting the sebaceous glands. To evaluate the anti-lipogenic effect, sebocytes were treated with test materials and ^{14}C-acetate incorporation assay was performed.
Key findings: To screen the anti-lipogenic materials, we tested the effect of many herbal plant extracts. We found that *Angelica dahurica* extract inhibited the insulin-like growth factor-1 (IGF-1)-induced sebum production in terms of squalene synthesis in sebocytes. Furthermore, imperatorin isolated from *A. dahurica* showed remarkable inhibitory effect on squalene production as well as squalene synthase promoter activity. To investigate the putative action mechanism, we tested the effect of imperatorin on intracellular signaling. The results showed that imperatorin inhibited IGF-1-induced phosphorylation of Akt. In addition, imperatorin significantly down-regulated PPAR-γ and SREBP-1, the important transcription factors for lipid synthesis.
Significance: These results suggest that imperatorin has a potential for reducing sebum production in sebocytes, and can be applicable for acne treatment.

1. Introduction

Acne is a common skin disease, which is originated from pilosebaceous unit consisting of a hair follicle and its accompanying sebaceous gland. Pathophysiology of acne is described as follows; 1) excess sebum production, 2) hyperkeratinization of pilosebaceous duct, 3) hyperproliferation of commensal *Propionibacterium acnes* (*P. acnes*), and 4) perifollicular inflammation [1]. For treatment of acne, many drugs have been developed targeting multiple action points. For example, antibiotics targeting commensal bacteria include tetracycline, doxycycline and minocycline [2]. Disadvantage of this targeting is the occurrence of drug-resistant strains [3,4]. Drugs that normalize pattern of follicular keratinization, such as adapalene, isotretinoin and tazarotene, are also widely used, however their use is highly regulated because of adverse effects [2,5]. There is still a high, unmet clinical need for new and better treatments not only with anti-lipogenic and anti-inflammatory actions but also with no or a lower propensity to cause side effects. In this regard, targeting the sebum production in sebaceous gland is one promising approach to develop novel compounds for acne treatment.

Medicinal plants are an important source for development of therapeutics for acne. Many active materials have been isolated from plant extracts, and reported that they have anti-acne effect. For example, ethanol extract of *Syzygium jambos* and one of the isolated compound anacardic acid analog inhibit the growth of *P. acnes* [6]. In other example, green tea ingredient epigallocatechin-3-gallate (EGCG) suppresses insulin-like growth factor-1 (IGF-1)-induced lipogenesis and cytokine expression in SZ95 sebocytes [7]. In a preliminary study, we attempted to find anti-lipogenic materials using medicinal plants, and found that imperatorin isolated from *Angelica dahurica* has such an effect. Imperatorin is a phytochemical classified as furocoumarin, which is consisted of furan ring and coumarin. It has been shown that imperatorin has anti-cancer potential and anti-hypertensive effect [8,9]. However, the effect of imperatorin on lipogenesis in sebocytes remains unclear. In

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this study, we demonstrate that imperatorin inhibits IGF-1-induced sebum production in terms of squalene synthesis in human sebocytes, suggesting that imperatorin may be developed for acne treatment.

2. Materials and methods

2.1. Cell culture

Human skin tissues were obtained under the written informed consent of donors, in accordance with the ethical committee approval process of the Institutional Review Board of Chungnam National University Hospital. Skin tissues were sterilized in 70% ethanol for 1 min, then sebaceous glands were dissected under the stereomicroscope. Isolated sebaceous glands were attached to the culture dish, and incubated in Sebomed® medium (Biochrom, Berlin, Germany) supplemented with 10% fetal bovine serum (FBS) and 5 ng/ml recombinant human epidermal growth factor (rhEGF) (Life Technologies Corporation, Grand Island, NY). Generally, sebocytes were outgrown from explanted sebaceous gland tissues in 1 week. Sebocytes at 3rd–7th passage were used for all experiments. In this study, we provide the data from sebocytes that were obtained from just one donor. The gender of donor is male.

2.2. A. dahurica extract and imperatorin

The methanol extract of root of *A. dahurica* was obtained from a plant extract bank at Korea Research Institute of Bioscience & Biotechnology (Daejeon, Korea). Imperatorin was purchased from ChromaDex (Irvine, CA), and the structure was confirmed by NMR spectroscopy. The plant extract and imperatorin were dissolved in dimethyl sulfoxide (DMSO).

2.3. Lipogenesis assay

For measurement of lipogenesis, a well-established ^{14}C-acetate incorporation assay was employed [10]. Cells were treated with test material for 2 d. Cells were then incubated with medium containing 2 μCi of [1-^{14}C]acetic acid sodium salt (PerkinElmer, Boston, MA) for 6 h. After washing twice with phosphate-buffered saline (PBS), cells were harvested and lipids were extracted with chloroform and methanol (2:1). The solvents were evaporated in fume hood overnight, and lipids were reconstituted in chloroform. Lipids were then separated using a thin layer chromatography (TLC silica gel 60 F_{254}, Merck KGaA, Darmstadt, Germany) with developing buffer consisting of hexane and ethyl acetate (6:1). Lipids were visualized by autoradiography.

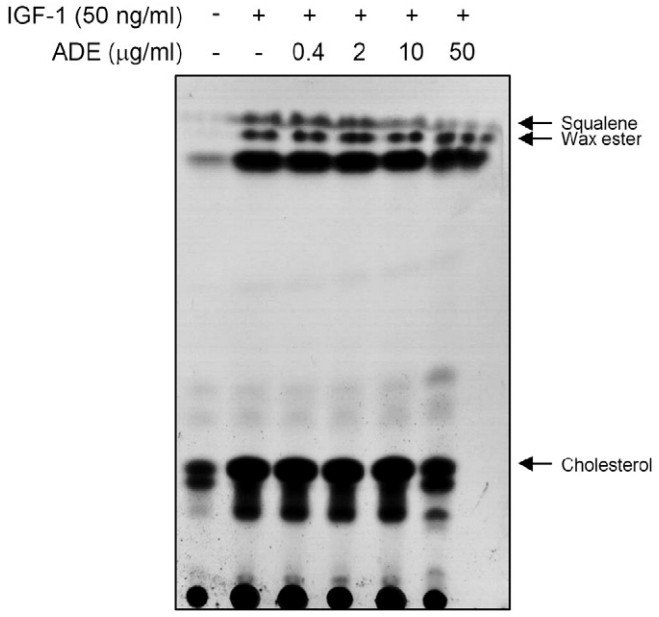

Fig. 1. Screening of anti-lipogenic materials. Sebocytes were cultured and lipogenesis was induced by insulin-like growth factor-1 (IGF-1). Effect of *Angelica dahurica* extract (ADE) on IGF-1-induced lipid production was determined by thin layer chromatography. Production of squalene, a unique sebum lipid, was inhibited by *Angelica dahurica* extract.

2.4. Cell viability test

For viability test, human sebocytes were seeded in 6-well plate at a density of 2×10^5 cells/well, treated with imperatorin for 2 days. After treatment, cells received 2 mg/ml MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution and were incubated for a further 4 h. The medium was removed and the resulting formazan crystal was solubilized in 100 μl of DMSO. The optical density at 540 nm was determined using an ELISA reader.

2.5. Nile red staining

Sebocytes were grown on cover glass and treated with imperatorin. Cells were fixed with 10% formalin for 5 min, incubated with Nile red solution for 15 min, then vigorously washed with PBS on a rocking platform. Cells were then observed under fluorescent microscope.

2.6. Luciferase reporter assay

For creation of squalene synthase-luc reporter adenovirus (Ad/SS-luc), genomic DNA isolated from sebocytes was used as a template for PCR. Primer sequences were as follows: squalene synthase promoter, 5′-GGTACCGAGCCCTGTGGCAGTTAGAG and 5′-AAGCTTCGACTTTCAC CTCTGCGGTG. The resultant PCR fragment covered from −3436 to −29 base pairs of squalene synthase translation start site. The promoter fragment was subcloned into pGL3 vector (Promega, Madison, WI), then moved to pENTR vector. The replication-incompetent adenovirus was created using Virapower™ adenoviral gateway expression kit (Life Technologies Corporation).

For luciferase reporter assay, sebocytes were seeded in 6-well plate, then transduced with 10 multiplicity of infection (MOI) of Ad/SS-Luc for 6 h. Cells were replenished with fresh medium and treated with imperatorin for 2 d. Luciferase activity was measured using the dual luciferase reporter assay system (Promega).

2.7. Western blot

Sebocytes were lysed in Proprep solution (Intron, Daejeon, Korea). Total protein was measured using a BCA Protein Assay Reagent (Pierce

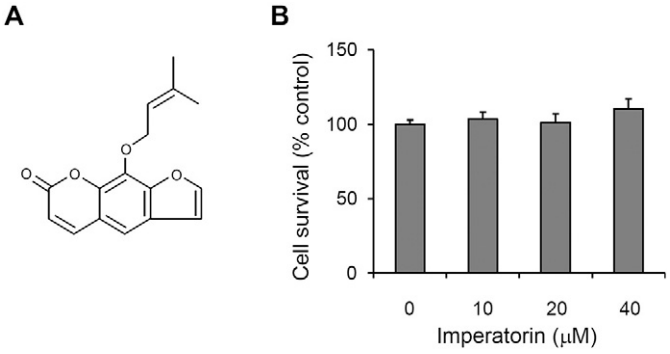

Fig. 2. (A) Structure of imperatorin, the main component of *Angelica dahurica*. (B) Cytotoxicity of imperatorin. Sebocytes were treated with imperatorin at the indicated concentrations for 2 days. Cell viability was measured by MTT assay. The mean values ± SD are averages of triplicate measurements.