STUDIES OF ACNE TREATMENT USING ORIENTAL HERBS

(New Approach to select anti-acne agents)

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Abstracts

Acne vulgaris, the most common skin disease, can be formed as only a few comedons or severe inflammatory lesions. The pathogenesis of acne involves various factors; excessive androgen, excessive sebum production, abnormal alteration of follicular epithelium, proliferation of *Propionibacterium acnes*, and inflammation. We investigated acne therapy using oriental herbs described in the Korean traditional medical book (Dong-ui-bo-gam). Oriental herbs (*Angelica daurica, Arctium lappa, Coptidis rhizoma,* and *Glycyrrhiza glabra*) were chosen based on their respective property of sebum control, anti-inflammatory activity, and anti-bacterial activity. We examined the effect of acne treatment, in terms of chemotactic inhibition, lipogenesis inhibition, and anti-bacterial activity for *P. acnes*. 1. Neutrophil chemotaxis assay; *P. acnes* secrete chemotactic factors and other pro-inflammatory extracellular products. Neutrophil chemotactic activity of *P. acnes* was measured by 48-well chemotaxis method. *Angelica daurica* clearly suppressed chemotactic activity of *P. acnes*. 2. Using sebaceous gland of hamster ear lipogenesis assay; Sebaceous lipogenesis was measured using ear biopsies by incubation of C^{14}-acetate in culture media. The C^{14}-labeled lipids were extracted and determined by liquid scintilation counting. *Coptidis rhizoma* markedly inhibited sebum production. 3. Anti-bacterial assay for *P. acnes* (MIC test); *Glycyrrhiza glabra* showed anti-bacterial activity. *P. acnes* did not develop resistance against *Glycyrrhiza glabra*.

Retinoids are effectively to inhibit sebum production and regulate follicular keratinization process, with little anti-inflammatory activity. *Angelica daurica* suppressed neutrophil chemotaxis, *Coptidis rhizoma* inhibited sebum production, and *Glycyrrhiza glabra* showed anti-bacterial activity against *P. acnes*. A combined formulation of *Angelica daurica, Coptidis*

rhizoma, and Glycyrrhiza glabra is expected to provide effective acne treatment.

Key words: acne, chemotactic inhibition, lipogenetic inhibition, antibacterial activity

Introduction

Acne is the most common skin disease, affecting nearly 80% of young adults[1]. The pathophysiologic factors that cause the development of acne are 1) excessive sebum production, 2) abnormal desquamation of pilosebaceous follicle epithelium (comedogenesis), and 3) proliferation of *Propionibacterium acnes* that result in inflammation[2].

Sebaceous glands and Sebum

Sebaceous gland activity is dependent on androgenic hormones. Sebum is produced by sebaceous gland, in part for the maintenance of epidermal hydration. Excessive androgen hormone results in overproduction of sebum[3].

Testosterone is converted to dihydrotestosterone, which binds to a high-affinity specific cytoplasmic receptor protein that is transported to the cell nucleus. At this point, the dihydrotestosterone-protein complex initiates DNA-controlled events[3].

As sebum secretion increases, in acne patients, corresponding decrease is observed in linoleic acid, wax esters, triglycerides, and free fatty acids of skin surface lipids[4]. Follicular epithelium differentiation may be influenced by the low concentration of linoleate in the sebum of acne patients[3].

Comedogenesis

Comedogenesis is an abnormality in the desquamation process of follicular corneocytes in the sebaceous follicular ducts. The microcomedo evolves into either a clinically apparent non-inflammatory lesion (an open comedo, "black head"), a closed comedo ("white head"), or inflammatory lesion, if *P. acnes* proliferate and generate inflammatory mediators[5] . Comedogenesis is not the result of abnormal keratinization, except for the presence of abnormal

lipid inclusions[6,7].

A number of theories have been offered to explain abnormal keratinization of pilosebaceous follicle. Among them is 1) Reduction of linoleic acid in sebum of acne patients and deficiency of essential free fatty acid result in hyperkeratinization[8], 2) In epidermis of acne patients, ceramide reduction induces decreasing of barrier function, or 3) The oxide of squalene is a possible factor in comedogenesis. Ultraviolet irradiation enhances the comedogenic property of squalene by increasing the amount of squalene peroxides as well as by forming fatty acid peroxides[9]. *P. acnes* is not essential for the initiation of comedogenesis[10].

Proliferation and Inflammation

Acne inflammation comes along with the development of inter-cellular and intracellular edema (spongiosis) of the follicular epithelium as resulting from exudation of a perivascular infiltrate. The prominent cells are lymphocytes (particularly T-helper cells) or polymorpho-nuclear leukocytes in the infiltrate[11]. Cornified cell, lipids, hair, and bacteria are deposited into the dermis, producing a non-immune-type inflammation.

The cutaneous flora consist of aerobic cocci *Staphylococcus epidermis*, the yeast *Pityrosporum ovale*, *Pityrosporum orbiculare*, and anaerobic diphtheroid *Propionibactrrium acnes*. Among them, the anaerobic diphtheroid *P. acnes* appears to play a central role in the development of acne inflammation.

The "free fatty acid hypothesis" is one of the first explanations offered for the acne inflammation. 1) There was a decrease in the percentage of free fatty acids in skin surface lipids with successful antibiotics therapy, and 2) a large proportion of those free fatty acids resulted from hydrolysis of sebaceous gland triglycerides by *P. acnes* [12,13]. Intradermal injection of free fatty acids into human volunteers resulted in intense inflammation[14]. This hypothesis assumes that free fatty acids can sufficiently irritate the follicular epithelium to result in its breakage, and can thereby enable fatty acids to penetrate the dermis and cause inflammation.

With the disruption of the follicular epithelium, sebaceous lipids, hair, *P. acnes*, and

cornified epithelial cells are extruded into the dermis, causing inflammation. Lipids, hairs, and cornified cells elicit then non-immune defense reactions associated with mononuclear cells, and then macrophages and giant cells. *P. acnes* generates a series of immune and nonimmune inflammatory reactions. The ingestion of *P. acnes* by polymorphonuclear leukocytes results in the release of hydrolases, which consequently causes further tissue destruction. *P. acnes* activates both the classic and alternative pathways of complement to produce C5-derived (C5a) neutrophil chemotactic factor, which in turn causes the ingress of polymorphonuclear leukocytes and inflammation, while simultaneously enhancing the release of hydrolytic enzymes from neutrophils[15,16,17]. Therefore, in treatment of acne, inhibition of comedogenesis and chemotaxis is important for the intervention of inflammation.

The aim of this investigation was to study the effects of oriental herbs with new strategy to screen anti-acne agents.

Materials and Methods

Inhibition of *P. acnes* Chemotaxis

P. acnes culture.

The *P. acnes* ATCC 6919 was cultured anaerobically on BHI agar for 5 to 7 days. A suspension in phosphate-buffered saline (PBS) was prepared from this culture. Two milliliters of this suspension was transferred aseptically to a 500 ml culture flask containing 300 ml of freshly prepared BHI media. The flask was then flushed with nitrogen and incubated at 37°C for 72 hrs. Once the culture was cooled immediately to 4°C, aliquot was removed and plated to determine cell number and to verify purity. The remainder of culture medium was centrifuged at 5000 × g for 15 min at 4°C. The supernatant was removed, filtered (through 0.22 µm pore-size filter), and used immediately or stored at -20°C. The bacteria pellet was washed three times in 100 ml of PBS, suspended 10 ml in PBS, sonicated bacteria solution, and finally made 1.0% *P. acne* sonicated solution (chemotactic solution).

PMN isolation

Blood was collected from normal rat abdominal artery using syringe. The heparinized blood was diluted to 1:2 with PBS (pH 7.2) and layered on a Ficoll-Paque® gradient (Pharmacia Biotech, Sweden). The gradient was centrifuged at 1200 rpm for 40 min at room temperature, removed peripheral blood mononuclear cells, and collected the polymorphonuclear cells (PMNs) and rich RBCs. PMNs and RBCs leaved 1.5% dextran solution for 20 min. PMNs and poor RBCs were washed three times in 0.2% saline and then is 1.6% saline. PMNs were washed twice in PBS.

Neutrophil chemotaxis inhibition assay[18]

Neutrophil chemotactic activity was measured using a 48-well micro chemotaxis. Briefly, 30 µl of chemotactic solution in RPMI1640 was placed in the bottom well. Dilutions were prepared in microtiter plates. A filter sheet was picked up by forceps and carefully placed on the bottom plate. Gasket and top plate were slipped over the bottom plate and bolted down. The PMN suspension (30 µl) was added into the top chambers and the apparatus was incubated for 1 hr. To remove non-migrated cells from the top slide of the filter at the end of incubation, the chamber was disassembled and the filter suspended between 2 clamps. The filter was then drawn gently up the edge of a windshield wiper blade. This was done twice with PBS washing between wipings. The filter was fixed for 5 min in methanol, mounted on a glass slide and dried. The cells on the mounted sheet were stained with Giemsa solution. Migrated cells on the filter sheets were counted 5 yields by the microscopy, ×400. The result was expressed as (total number of migrated cells/number of selected area).

Antibacterial activity against *P. acnes* (MIC test, Broth dilution test)
MIC test, Broth dilution test[19]

Antibacterial activity against *P. acnes* was evaluated by medium dilution method. Overnight cultures of 2 strains of *P. acnes* (ATCC 6919, 11827) were diluted to a density of 1×10^6 colony-forming units (CFU)/ml in BHI medium and inoculated by a multiple inoculum replicator into the test tubes containing 2-fold dilutions of materials. The MICs of these materials

were determined after anaerobic incubation at 37°C for 48 hrs.

P. acnes resistance against Glycyrrhiza glabra and Erythromycin against

P. acnes with methanol extract of Glycyrrhiza glabra and erythromycin was measured with subculture each MIC. If MIC was over 500 ppm, then P. acnes is considered to develop resistance.

$$AI \text{ (adaptation index)} = \frac{\text{MIC after subculture}}{\text{First MIC}}$$

Lipogenesis of sebaceous glands[20]

Adult male Syrian hamsters (5-6 weeks) were used. $CH_3^{14}COONa$ was used as a labeled precursor for the lipogenesis study and $C(^3H)H_2COONa$ for autoradiography. Eagle's minimum essential medium, containing 10% fetal bovine serum and antibiotics, was used as the incubation medium. $CH_3^{14}COONa$ was added as a labeled precursor. Oriental herbs (Angelica daurica, Arctium Lappa, Coptidis rhizoma, and Glycyrrhiza glabra) were added to each medium to give 0.01% in the experimental group. $C(^3H)H_2COONa$ was used instead of $CH_3^{14}COONa$ for autoradiography study to confirm the incorporation of labeled acetate into the sebaceous glands.

The incorporation of ^{14}C-acetate into lipids was studied in the following way : hamsters were sacrificed by cervical dislocation, and 6-mm punch biopsies were taken from the ventral surface of the ear lobes. The underlying cartilage and connective tissues were removed from the biopsied discs with blunt forceps. The specimen was floated, with the epidermal side up, in 2 ml of medium (the specimens from the right ear's specimens in the medium containing oriental herb extract and those from the left ear's specimens in the control medium) and incubated by shaking in a CO_2 incubator for 6 hrs at 37°C. After incubation, the specimens were removed from the medium and washed twice with saline. Lipids were extracted from dermis with 1 ml of a chloroform-methanol (2:1, v/v) solution for 12 hrs at room temperature. The amount of ^{14}C in 0.9 ml of the lipid extracts was counted in a liquid scintillation counter using a toluene-based scintillation cocktail. Radioactivity was expressed by the mean ± S.D. counts per minute (dpm).

Results

Inhibiton of *P. acnes* Chemotaxis

Angelica daurica (0.01%) was effectively inhibited chemotaxis of *P. acnes*, at the same level erythromycin did. *Angelica daurica* (0.01%) inhibited 75% chemotatic activity of *P. acnes*.
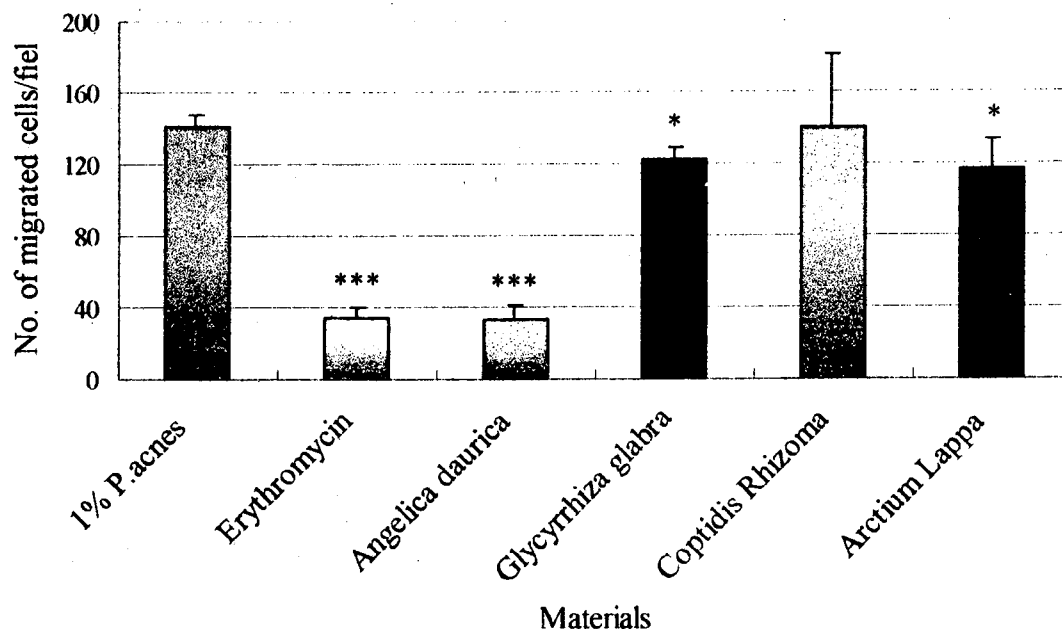

Fig 1. Inhibition of *P. acnes* Chemotaxis by oriental herbs. The results are mean ± S.D. *p<0.05, ***p<0.001 were statistically significant.

Antibacterial activity against *P. acnes* (MIC test, Broth dilution test)

Table 1. Minimum inhibitory concentration (MIC) test against *P. acnes*

Materials	MIC (ppm, μg/ml)	
	P. acnes ATCC 6919	*P. acnes* ATCC 11827
Erythromycin	300	0.5
Extract of *Angelica daurica*	1700	1850
Extract of *Coptidis rhizoma*	1050	1100
Extract of *Glycyrrhiza glabra*	200	100
Extract of *Arctium lappa*	1400	1400

Glycyrrhiza glabra was showed anti-bacterial activity for *P. acnes* ATCC 6919, as much as erythromycin did. Also, *Glycyrrhiza glabra* showed anti-bacterial activity for *P. acnes* ATCC

Resistance test of *Glycyrrhiza glabra* and Erythromycin against *P. acnes*

Table 2. Resistance test against *P. acnes*

Materials	Changes of MIC and AI							
	ATCC 6919				ATCC 11827			
	C1	C2	C3	AI	C1	C2	C3	AI
Glycyrrhiza glabra	200	600	600	3	100	400	400	4
Erythromycin	300	1200	2500	8.2	0.5	2	200	400

Glycyrrhiza glabra didn't obtain resistance against *P. acnes*, but erythromycin obtained resistance against *P. acnes*.

Lipogenesis of sebaceous glands

Lipogenesis was suppressed 84.9% and 16.7% by 0.01% *Coptidis rhizoma* and 0.01% *Arctium lappa*, respectively (Fig 2).

Decision and Discussion

Angelica daurica (Baek-jee)[21,22]

Angelica daurica is 1- 1.5 meters high, a kind of perennial plant. Its fruit is small rounded, and its root is called Baek-jee, which composed of imperatorin, phellopterin, xantoroxin, byakangelcol, oxypeucedanin, neobyakangelcol (coumarin components, 0.3-0.9%). Procoumarin has effect on painkiller and sedation.

Arctium lappa (Woo-eong)[21,22]

Arctium lappa is 1-2 meters high, a kind of perennial plant. Its fruit is composed of lignan glucoside, oil 25-30%, phytosterin, Vitamin B1, saponin, coumarin, lemonic acid, malic acid. Its root is composed of inulin 27-45%, protein, oil, arctein, caffeic acid, tannin, alkaloids. Its fruit has effect of diuresis, anti-inflammation, and pyorrhea. Its root has effect of diuresis and
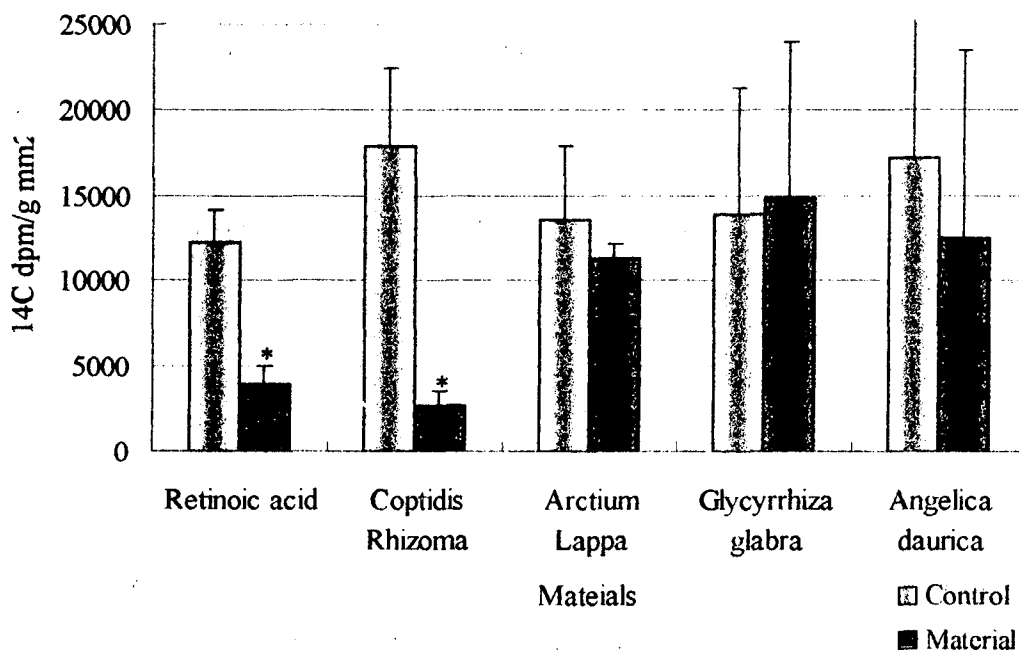
metabolic enhancement.

Fig 2. Anti-lipogenic activity of oriental herbs. The results are mean ± S.D. *p<0.05 was statistically significant.

Coptidis rhizoma (Hwang-lyeon)[21,22]

Coptidis rhizoma's drided root and stem contain berberine. Its water extract has effect on lipase activation and pepsin inhibition. Its methanol extract has anti-inflammatory and preventive effects of arteriosclerosis.

Glycyrrhiza glabra (Gam-cho)[21,22]

Glycyrrhiza glabra has specific odor, and sweet taste. Its components are triterpene glycoside, glabric acid, flavanone, isoflavon etc. Glycyrrhizine (genin), a main component, has effect; estrogen stimulating activity, anti-inflammation, and poison neutralization.

Angelica daurica suppressed neutrophil chemotaxis, as much as erythromycin did. *Coptidis rhizoma* effectively inhibited sebum production perhaps due to berberine. Berberine inhibited generation of triglycerides[20]. *Glycyrrhiza glabra* showed anti-bacterial activity for *P.*

acnes and didn't show resistance against *P. acnes*.

We expect that a combined formulation of *Angelica daurica, Coptidis rhizoma, and Glycyrrhiza glabra* and some keratolytic agent has a great potential for acne treatment.

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