Cryptotanshinone for Treating Acne Vulgaris

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Abstract

Tests of stability and toxicity, and clinical evaluation of anti-acne activity suggest that cryptotanshinone, a constituent of the roots of *Salvia miltiorrhiza* Bunge, is an effective active ingredient for acne vulgaris treatments.

Acne vulgaris, called acne or pimples, is the most common disease of the pilosebaceous follicle unit of the skin. It affects nearly 80% of people between the ages of 11 and 30. Approximately 30% of teenagers have acne of sufficient severity to require medical treatment.

Acne is a follicular disorder of the skin. It occurs in specialized pilosebaceous units on the face and body. Acne develops when these specialized follicles undergo pathologic alterations that result in the formation of non-inflammatory lesions (comedones) and inflammatory lesions (papules, pustules and nodules). An abnormality of keratinizing epithelium of these follicles, thought to be due to the action of sebum synthesized and secreted by the androgen-sensitive sebaceous glands, leads to inflammation induced by the follicular bacterium *Propionibacterium acnes*.

Therapy involves treatments that modify these pathogenic factors and includes drugs with antikeratinizing, antibacterial and antiseborrheic actions. Acne vulgaris is a very frequent disease, seen primarily in adolescents, involving the sebaceous follicles. Acne vulgaris is characterized by a great variety of clinical inflammatory and non-inflammatory lesions: comedones, papules, pustules, nodules, cysts and scars. Acne vulgaris is a multi-factorial disease. Although its pathogenicity is unclear, extensive studies have shown that hyperseborrhea, superinfection by *P. acnes* and endocrinologic androgenic changes play a role in the development of acne vulgaris.

Introduction

"Danshen," the root of *Salvia miltiorrhiza* Bunge (Labiatae), is one of the most important medicinal herbs, and has been widely used in China and Korea for the treatment of disorders such as hemorrhage, menstrual disorder, miscarriage, hepatitis, heart disease, edema, and swelling.[1]

More than 20 diterpene quinones known as tanshinones have been isolated from this herb, and examined for their inhibitory activity on the growth of bacteria and fungi. Fang et al.[2] showed that the Danshen root's orange-red pigments such as cryptotanshinone, dihydrotanshinone I, tanshinone IIA, methylenetanshinoate, and tanshinone IIB were inhibitory to the growth of *Staphylococcus aureus* cultured in vitro. Zheng and Ho[3] also reported that all five varieties of Danshen were active against gram-positive bacteria. The five compounds have mutually similar chemical structures (Figure 1).

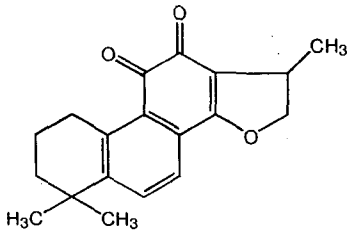

Figure 1. Chemical Structure of Cryptotanshinone

We had studied the stability, skin sensitizing potential and anti-bacterial activity of these five chemical compounds of Danshen: cryptotanshinone, dihydrotanshinone, tanshinone IIA, methylenetanshionate and tanshinone I. In that study, cryptotanshinone had a better anti-bacterial activity, stability against UV and heat and a lower skin sensitizing potential than other compounds.

The antibacterial activity against *P. acnes*, 5α-reductase inhibition leading to a decreased sebum production and comedolytic effects are the important pharmacological target sites of anti-acne products. Our studies showed that cryptotanshinone displayed antibacterial activity toward *P. acnes* and *S. aureus* , antiseborrheic property potential by showing the inhibition of *5α-reductase activity* and anti-inflammation activity. These results prompted us to test the efficacy of this compound against mild acne vulgaris

Methods and Materials

Materials: Arachidonic acid and indomethacin were purchased from Sigma Co. (St. Louis, MO, USA). Stearyl glycyrrhetinate was purchased from Maruzen (Japan). DMEM (Dulbecco's Modified Ea□□s M

Medium), fetal bovine serum (FBS), streptomycin and penicillin were all purchased from GibcoBRL (Grand Island, NY, USA). [^{3}H] Methyl thymidine (^{3}HTdR) and [1, 2, 6, 7-^{3}H] testosterone were from Amersham (USA). Potassium phosphate, sodium chloride and sodium phosphate were obtained from Sigma Co. (USA). HPLC grade methanol, methylene chloride and acetonitrile were all purchased from J. T. Baker Chemical Co. (Muskegon, MI, USA). Triethylhexanoin was obtained from Kokyu Alcohol (Japan). All other ingredients were reagent or cosmetic grade.

Triethylhexanoin solution containing 0.1% (w/w) cryptotanshinone was prepared for the UV stabilities. A solubilized state (Formula 1) was prepared for anti-microbial activity test and an o/w emulsion (Formula 2) containing 0.1% of cryptotanshinone was prepared for stability studies. This o/w emulsion also used for toxicity and clinical tests.

Formula I. Cryptotanshinone in solubilized state for anti-bacterial activity	
POE (40) Hydrogenated caster oil	1.0%
Cryptotanshinone	0.1
Ethanol	10.0
Water (aqua)	q.s 100
Formula 2. Cryptotanshinone in o/w emulsion for stability studies	
Stearyl alcohol	0.8%
Behenyl alcohol	0.8
Glyceryl stearate SE	1.0
Glyceryl stearate (and) PEG-100 stearate	1.5
Squalane	9.0
Triethylhexanoin	4.0
Polysorbate 60	1.5
Sorbitan stearate	0.3
Cryptotanshinone	0.1
Propylene glycol	7.0
TEA	0.15
Carbomer	0.12
Water (aqua)	qs 100.00

Preparing cryptotanshinone : A powdered sample (2.5 kg) of Danshen purchased at a crude drug market in China was extracted with methanol (4 × 6ℓ) for 3 hr, and the methanol was evaporated under reduced pressure to obtain a syrup extract (413g). When the extract was distributed between ether and water layers, the anti-bacterial activity against *P. acnes* was found only in the former, ether layer (43g), which was then subjected to silica gel column (Mallinckrodt, 900g) chromatography using $CHCl_3$ as the developing solvent. Each eluted fraction (300 ㎖) was examined by the paper disc method for anti-bacterial activity against *P. acnes*.

Cryptotanshinone (826 ㎎) was isolated as main active compound by preparative TLC using silica gel G60 (developing solvent: CH_3Cl). In addition, tanshinone IIA and tanshinone I were obtained as crystal with same method respectively, but they were found to be inactive against the bacteria.

The characterization of the cryptotanshinone was carried out by melting point determination, elementary analysis, ultraviolet (UV), infrared (IR), proton nuclear magnetic resonance (^{1}H-NMR), and mass spectrum (MS).[4]

Local lymph node assay : The murine local lymph node assay (LLNA) can be used to determine the relative skin sensitizing potency of chemicals via interpolation of the quantitative dose response data generated.[5] Using this approach, we examined the skin sensitizing potency of five Danshen compounds: cryptotanshinone, dihydrotanshinone, tanshinone IIA, methylenetanshinone and tanshinone I. The standard LLNA protocol was used as described previously.[6]

Test mice were exposed topically on the dorsum of both ears to test solutions (5, 10, 20%) of the Danshen active compound in a 4:1 (v/v) acetone : olive oil vehicle. Control mice were similarly exposed to the vehicle only. For each concentration of the test chemical, a stimulation index (SI) was derived using the value obtained from the concurrent vehicle control group as the comparator.

The policy currently is to consider as skin sensitizers those chemicals that, at one or more concentrations, provoke a threefold or greater increase in lymph node cell proliferative activity compared with concurrent vehicle. In other words, sensitizers would be chemicals that induce an SI of 3 or more.[6]

Determining MIC of cryptotanshinone and benzoyl peroxide : The MICs of cryptotanshinone and benzoyl peroxide were determined on nutrient agar plates and carried out by agar dilution.[7] Quarter-strength brain heart infusion agar (Difco Laboratories, East Molesey, Surrey, UK) containing 0.75g/㎖ additional glucose (BHIg) was used for the propionibacteria and staphylococci.

Propionibacterial inocula was derived from cultures incubated for 2 days anaerobically in full-length BHIg broth and staphylococcal inocula from cultures incubated overnight aerobically in full-length BHIg broth, both at 37□□. The cryptotanshinone test sample was prepared in solubilized state in Formula 1 and the control sample was prepared at the same condition except for the absence of cryptotanshinone. Benzoyl peroxide was prepared at the same condition.

Inocula were diluted to an optical density of 0.2 at 600 ㎚, equivalent to ~10^8 colony forming units (cfu) per ㎖, before delivery to plates via multipoint inoculator to give a final inoculum density of 10^5 cfu per spot. Test plates were incubated at 37□□, 3 days anaerobically for the propionibacteria, and aerobically overnight for the staphylococci. MICs were recorded as the lowest dilution concentration of each chemical for each organism yielding no growth or a barely visible haze, as determined with the unaided eye.

Enzymatic assays of 5α-reductase activity : The effect of restraining sebum secretion was verified by the following experiment about the effect of cryptotanshinone inhibiting 5α-reductase from being active. 5α-reductase activity was measured as previously described. [8,9]

All procedure were carried out as rapidly as possible at 0-4□□ with ice-cold reagents. Crude 5α-reductase was prepared from female rat liver. Male Sprague-Dawley rats, 40 to 60 days old, were sacrificed by carbon dioxide asphyxiation. The livers were excised rapidly and washed three times with phosphate-buffered saline (PBS) and then placed in ice-cold homogenization buffer (50mM Na_2HPO_4, 0.25 M sucrose). Rat liver tissue in buffer was minced with scissors and homogenized with a Polytron (three 10s bursts at setting 4). The homogenate was forced through a wire screen (900mesh), and crude nuclear pellets and cytosol supernatant fractions were obtained after centrifugation at 3000X g for 10min at 4□□.

10 ㎕ of test concentration of the test chemical was incubated with 40 ㎕ of cytosol supernatant fractions in 50 ㎕ of reaction buffer [100mM PBS (at pH 6.6 for Type 1 reductase activity), 1mM dithiothreitol, 1mM NADPH and 259 nM [1, 2, 6, 7-^{3}H] testosterone] for 15min at 37□□. The reaction was stopped with 150 ㎕ of stop buffer (ethylacetate: cyclohexane 3:7, v/v) and stored at room temperature for 15min with agitation.

The amount of converted dihydrotestosterone is measured by HPLC equipped with radioactive measuring detector (LB 507B, EG&G Berthold, Germany). According to the following formula, the inhibition rate(%) of cryptotanshinone against a 5α-reductase type 1 is obtained.

$$\% \text{ inhibition} = \left\{ \frac{(A - B)}{A5} \right\} \times 100$$

A = conversion rate from testosterone to dihydrotestosterone with no test material

B = conversion rate from testosterone to dihydrotestosterone with test material

Evaluating anti-inflammatory effect of cryptotanshinone : The anti-inflammatory effect assay was based on the inhibition of arachidonic acid-induced ear edema as a model system for assessing topical anti-inflammatory compounds.[10] Arachidonic acid was dissolved in acetone and test compounds were dissolved in ethanol.

After washing both ears of mice with ethanol before applying the test compound, 20 $\mu\ell$ of compounds tested for their anti-inflammatory effect were topically applied to the each ear in an application group only once a day for four days, while 20 $\mu\ell$ of ethanol was applied to the each ear in the comparative group. One hour following the last application, ethanol was applied to the left ears of the mice and 2 mg/ear of arachidonic acid was applied to the right ears of the mice. One hour after that, the degree of ear edema was repeatedly measured (three times for each ear) by a dial thickness gauge calibrated with units of 0.01 mm. The left ear of mice in the application group was used as a comparative for measuring the degree of inflammation being decreased by the test compound. The left ear of mice in the comparative group was not used. Ear edema was expressed as the difference in ear thickness between test animals and controls.

This value was expressed as % of the full swelling, and hence a % inhibition by the test compound was calculated as follows:

$$\% \text{ inhibition} = \left\{ \frac{S_c - S_a}{S_c} \right\} \times 100$$

$$= \left\{ \frac{(x-y) - (a-b)}{(x-y)} \right\} \times 100$$

where

S_c = swelling in the comparative group

S_a = swelling in the application group

X = mean thickness of the right ears treated with arachidonic acid in comparative group

Y = mean thickness of the left ears not treated with arachidonic acid in comparative group

a = mean thickness of the right ears treated with arachidonic acid in application group

b = mean thickness of the right control ears not with arachidonic acid in application group

This method reduces the contribution of inter-animal variations in responsiveness to the inflammatory stimulus. The significance of the inhibition was calculated using a paired '*t*' test of the two values of proportional thickness increases.

Stability studies : To measure the temperature stabilities at various pH values, cryptotanshinone was prepared in o/w emulsion. Samples were stored at 25°C and 40°C°for as long as approximately 2 months. Periodically, 50 ㎖ aliquots of each sample were collected out and diluted with methanol. The amount of residual cryptotanshinone was measured by HPLC.

To measure the UV stabilities of cryptotanshinone, 1 g aliquots of o/w emulsion containing cryptotanshinone were added to each test tube. The samples were illuminated with UV from artificial light generator (Sun Test CPS, Heraeus Co., Hanau, Germany) for 60 min. The amount of residual cryptotanshinone was measured by HPLC.

Toxicity tests : We tested the toxicity of cryptotanshinone on animals *in vivo* using toxicity tests based on the Organization for Economic Cooperation and Development's 1960 guidelines for testing of chemicals. To estimate the toxicity, we performed tests on acute oral toxicity, acute transdermal toxicity, primary skin irritation, ocular irritation, skin sensitization and human patch according to the methods of Seo et al.[11]

Clinical test study design : To evaluate the efficacy of anti-acne activity, a full-face parallel groups design was selected. There were two test products. One was an o/w emulsion containing 0.1% cryptotanshinone and the other was placebo. 30 panelists were used to test each sample on the face.

Each test sample was applied twice a day (each morning and evening) for a period of 8 weeks. Evaluations were taken at the baseline, and during Week 8. Evaluation of global conditions (such as, number, size and color) of each kind of lesion were done through dermatological assessment, count of each kind of lesions in the target area, and impressions of target lesions taken with a self-assessment questionnaire. Skin safety parameters were also evaluated.

Subject selection is a critical factor and may be the most important one in planning the acne clinical trial. Subjects must be selected depending on the various requirements shown in Table 1.

Table 1. Guidelines for Selecting Subjects for an Acne Clinical Study

◇ Inclusion Criteria

① Males/Females in good health

② Age 14-30

③ With mild acne, more than 5 lesions on the face

◇ Exclusion Criteria

① Currently involved in another clinical investigation or participation in facial skin care and clinical investigation within the last 3 months.

② Pregnant or nursing female.

③ History of sensitivity to topical formulations or their components.

④ Having facial skin disorders (such as inflammation, eczema, psoriasis, lupus, skin cancer).

⑤ Currently or within last 6 months use(d) oral retinoids, antibiotics and/or steroids.

⑥ Currently or within last 3 months use(d) topical retinoids, alpha hydroxy acids, salicylic acid, hydroquinone or any whitening preparation.

Clinical evaluation of anti-acne activity : To obtain a clinical evaluation of anti-acne activity, we performed both a global assessment and a lesion counting.

To avoid the problem intrinsic to lesion counting, dermatologists developed adequate global evaluation scales that would allow the quick assessment of the acne condition. This global assessment, which evaluated both skin irritation reactions and each kind of lesion, was performed on the baseline and during Week 8 by an experienced dermatologist.[12] Overall severity score of acne was determined at each visit according to a semi-quantitative scale: 0 (normal), 1 (mild), 2 (moderate), 3 (severe). Skin side effect parameters (erythema, edema, dryness, peeling) were assessed only on the skin being treated.

Lesion count is the most commonly used assessment technique. The target lesion was chosen by topical area on the cheek. The counting area was 3.5×2.5 ㎝. The dermatologist periodically counted the different lesions (such as open comedones, closed comedones, papule and pustule) on the target lesion separately.

Results and Discussion

Evaluating skin sensitizing potential of Danshen tanshinones : In recent years, several efficient

compounds have been developed for the treatment of acne vulgaris. But the efficacy of these new potent compounds has been accompanied by a number of side effects (such as irritation and allergic contact dermatitis) limiting patient compliance with treatment.[13] Therefore, the skin sensitizing potential of compound is considered as one of very important checkpoints to develop an efficient active ingredient for acne vulgaris treatments.

The murine local lymph node assay (LLNA) was developed as a method for identifying chemicals that have the ability to cause skin sensitization and allergic contact dermatitis.[14] The results obtained from the LLNA are presented in Table 2, which shows the disintegrations per minute (dpm) node^{-1} and SI values for the five tanshinones from *Salvia miltiorrhiza*. At the concentrations tested, cryptotanshinone gave the lowest stimulation index and methylenetanshinone gave the highest, with values of 1.3-1.5 and 5.3-12.5, respectively.

Table 2. Local lymph node assay results of tanshinones

Compound/ Concentration (% w/v)	[^{3}H] Thymidine incorporation (dpm /node)	SI*
AOO	1409	1
Cryptotanshinone 5%	2536	1.8
Cryptotanshinone 10%	1832	1.3
Cryptotanshinone 20%	2114	1.5
Methylenetanshinone 5%	7446	5.3
Methylenetanshinone 10%	10785	7.7
Methylenetanshinone 20%	17713	12.6
Tanshinone I 5%	2958	2.1
Tanshinone I 10%	4013	2.8
Tanshinone I 20%	4730	3.4
Tanshinone IIA 5%	1409	1.0
Tanshinone IIA 10%	2818	2.0
Tanshinone IIA 20%	3100	2.2
Dihydrotanshinone 5%	1973	1.4
Dihydrotanshinone 10%	2677	1.9
Dihydrotanshinone 20%	2536	1.8

* SI= stimulation index (test group value/control group value)

Methylenetanshinone and tanshinone I showed a dose response relationship and at 20% concentration, they gave stimulation indexes of 12.6 and 3.4, respectively, classifying them as allergens leading to an allergic reaction and contact dermatitis.

Cryptotanshinone, tanshinone IIA, dihydrotanshinone induced an SI of 3 or less. Because the decision originally to use SI=3 as the criterion for distinguishing between sensitizing and non-sensitizing chemicals in the LLNA is arbitrary[14], cryptotanshinone, tanshinone IIA, and dihydrotanshinone are believed to be non-sensitizers. Shi et al.[15] suggested that cryptotanshinone achieves its potent anti-allergic activity by inhibiting mast cell degranulation.

Inhibition of bacterial growth by cryptotanshinone : The MICs of cryptotanshinone and benzoyl peroxide are shown in Table 3. The main microorganisms found in the sebaceous duct are an anaerobic propionibacteria and one or two species of staphylococci.

Table 3. Antibacterial activities of cryptotanshinone and benzoyl peroxide as measured by the serial agar dilution method

Bacterial strains	Testing material	MIC* (μg/mℓ)
Propionibacterium acnes ATCC 6919	Cryptotanshinone	100-500ppm
	Benzoyl peroxide	500-1000ppm
Staphylococcus aureus ATCC 6538	Cryptotanshinone	100-500ppm
	Benzoyl peroxide	500-1000ppm

* The minimum inhibitory concentration (MIC) values were found by the serial agar dilution method.

Under the conditions used, cryptotanshinone inhibited growth of *P. acnes* and *S. aureus* more effectively than benzoyl peroxide, which is often used for conventional acne treatment.[16] Therefore, application of cryptotanshinone will result in a healthy skin flora.

Another Danshen compound, dihydrotanshinone, also had a similar antibacterial activity against *P. acnes* and *S. aureus* (data not shown).[17]

Inhibition of 5α-reductase activity of cryptotanshinone : As illustrated in Table 4, the addition of cryptotanshinone led to a potent inhibition of type I 5α-reductase. Elevated levels of type I 5α-reductase

and its product, 5α-dihydrotestosterone are associated with a number of androgen-dependent skin condition such as acne, seborrhea by the development and enlargement of the sebaceous gland leading to the increase of sebum secretion level. Therefore, cryptotanshinone would be a potential therapeutic agent for the treatment of acne and seborrhea.

Table 4. Inhibition rate of testosterone 5α-reductase

Concentration of Cryptotanshinone (%)	Inhibition rate (%)
0.001	0
0.01	0
0.1	57

Anti-inflammatory effect of cryptotanshinone : The anti-inflammation effect is assessed by the degree that edema in the agent application group was restrained by cryptotanshinone, when compared to the edema reduction in the group to which arachidonic acid was applied.

As shown in Table 5, cryptotanshinone had a superior inflammation inhibition rate of 24.9%. This value is higher than that of stearyl glycyrrhetinate, known to have good anti-inflammation effect and generally used as an anti-inflammation agent in cosmetics. Cryptotanshinone's inflammation inhibition rate corresponds to about half that of indomethacin, a pharmacologically active ingredient having a superior anti-inflammation effect, with a significance level of 99%.

Table 5. Inhibition of the arachidonic acid-induced ear oedema by cryptotanshinone

Compound	Concentration (%)	Inhibition of oedema (%)
Cryptotanshinone	0.3	24.9*
Stearyl Glycyrrhetinate	0.3	15.8*
Indomethacin	0.3	46.6**

*p <0.05; **p <0.01 by paired 't' test (n=7), otherwise not statistically significant.

Stability studies : Figures 2 and 3 demonstrate the stabilities of cryptotanshinone in o/w emulsion at selected pHs during 2 months of storage at 25°C and 40°C. Cryptotanshinone was very stable at all tested pHs and apt to be more stable at lower pH than higher pH.

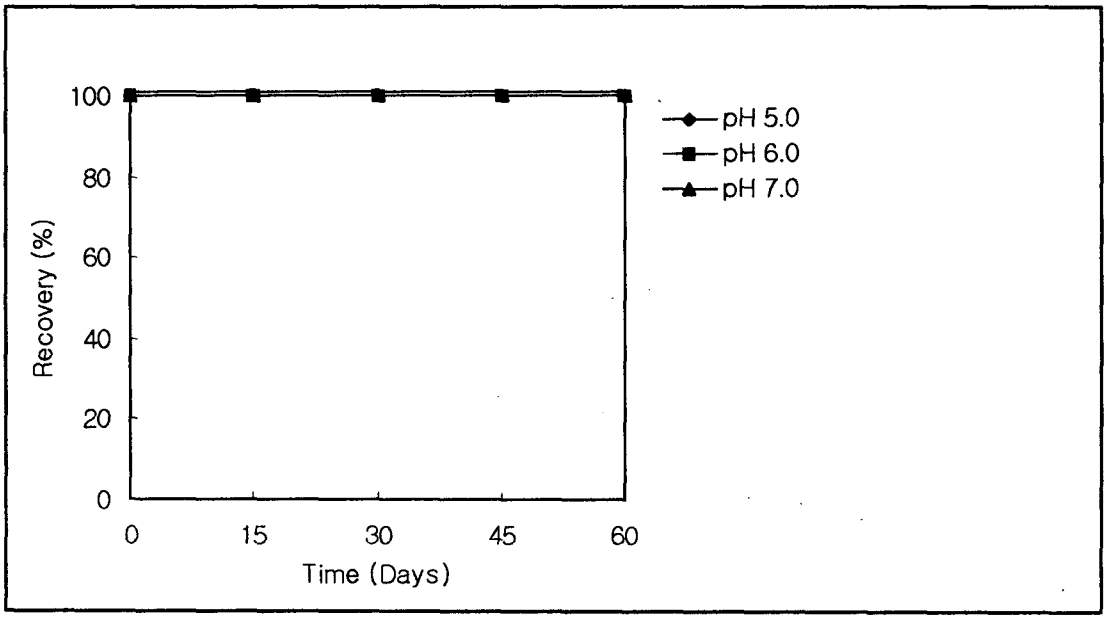

Figure 2. Recovery percent of cryptotanshinone in o/w emulsion stored at 25℃

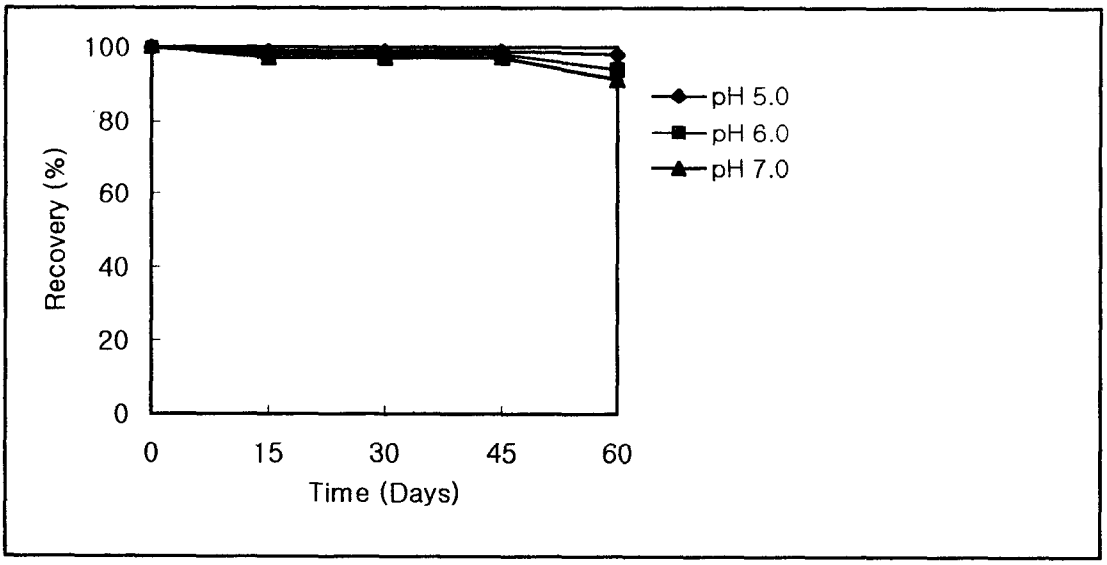

Figure 3. Recovery percent of cryptotanshinone in o/w emulsion stored at 40□□

Figure 4 shows stabilities of cryptotanshinone in triethylhexanoin and o/w emulsion under UV

treatment during 60 minutes. These results show cryptotanshinone was very unstable when exposed to UV. Therefore, the UV shield container must be used for cosmetic products containing cryptotanshinone.

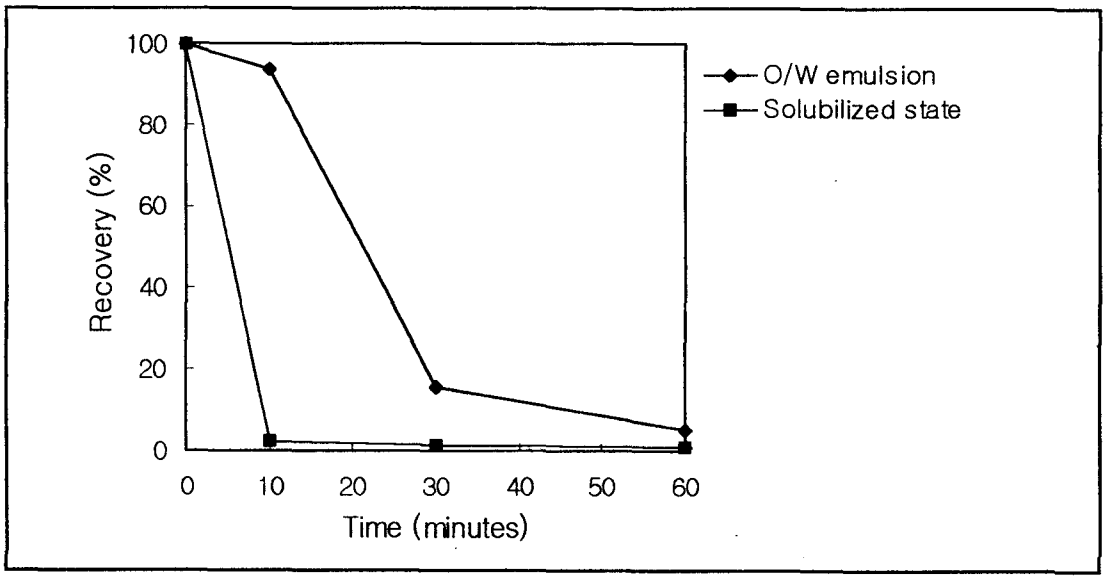

Figure 4. UV Stability of cryptotanshinone in O/W emulsion

Toxicity: To estimate the safety of cryptotanshinone, various safety tests were carried out. Cryptotanshinone was nontoxic in these tests, proving that it can be safely introduced to the skin care formulations.

◎ *Acute oral toxicity:* When we administrated the corn oil solution containing 0.1% cryptotanshinone orally in doses of 5,000, 2,500, 1,250, 625, and 312.5 ㎎/㎏ of body weight in healthy Sprague-Dawley rats, we observed neither unusual symptoms nor death.

◎ *Acute transdermal toxicity:* When we applied the dimethyl sulfoxide solution containing 0.1% cryptotanshinone in doses of 5,000, 2,500, 1,250, 625, and 312.5 ㎎/㎏ of body weight on the skin of selected healthy Sprague-Dawley rats, we observed no changes. Autopsies revealed no visual pathological symptoms.

◎ *Dermal primary irritation in rabbits:* We observed neither general symptoms nor weight change when the dimethyl sulfoxide solution containing 0.1% cryptotanshinone was applied to the skin. Nor did we see any erythema or formation of scale or edema at the application site. The P.I.I.(Primary Irritation Index by Draize) value was zero. Thus, there is no skin irritancy associated with the dimethyl sulfoxide solution containing 0.1% cryptotanshinone.

◎ *Ocular irritation in rabbits:* Neither general symptoms nor weight change were observed. There was no observable turbidity of cornea, abnormality of the iris, redness of the conjunctiva, edema or secretion. We concluded that cryptotanshinone, itself, does not induce eye irritancy in the New Zealand white rabbits.

◎ *Skin sensitization in guinea pig:* According to Magnusson and Kligman□s evaluating standard[8] the hypersensitivity score was zero and the hypersensitivity induction rate was 0%. Thus, cryptotanshinone causes no sensitized hypersensitivity in guinea pigs.

◎ *Human patch test:* When we applied the emulsion containing 0.1% cryptotanshinone, the Draize score was zero.

Clinical results from dermatologist assessment : The dermatologist global assessment results are shown in Table 6. The improvement rate of the o/w emulsion containing 0.1% cryptotanshinone is significantly higher ($p<0.05$) than that of the placebo at Week 8. The improvement in the severity of acne (reduced acne score) in the placebo group was probably due to the well-known seasonal variation of acne in temperate climate. Further controlled clinical studies performed during other seasons of the year might explain this.

Table 6. Clinical measurement of acne condition by the experienced dermatologist

Test Sample	Variable	Mean	SD	N
Placebo	A.S. Before	1.35	0.59	30
	A.S. After	0.95	0.65	30
	Improvement Rate	38.49	25.61	30
Cryptotanshinone 0.1%	A.S. Before	1.57	0.68	30
	A.S. After	0.74	0.54	30
	Improvement Rate	51.25	33.03	30

Clinical results from lesion counting : The different lesions were counted separately. The number of open comedones, closed comedones, papules and pustules were recorded at Day 0 and during Week 8.

In the most typical form of mild acne vulgaris, comedones predominate with occasional pustules. In

more severe cases, pustules and papules predominate. Figures 5 and 6 show that cryptotanshinone is more effective in reducing the number of open comedones and pustules in mild-to-moderate acne vulgaris. There was no significant difference between the 0.1% cryptotanshinone-treated groups in the reduction of closed comedones and papules when compared with the placebo group.

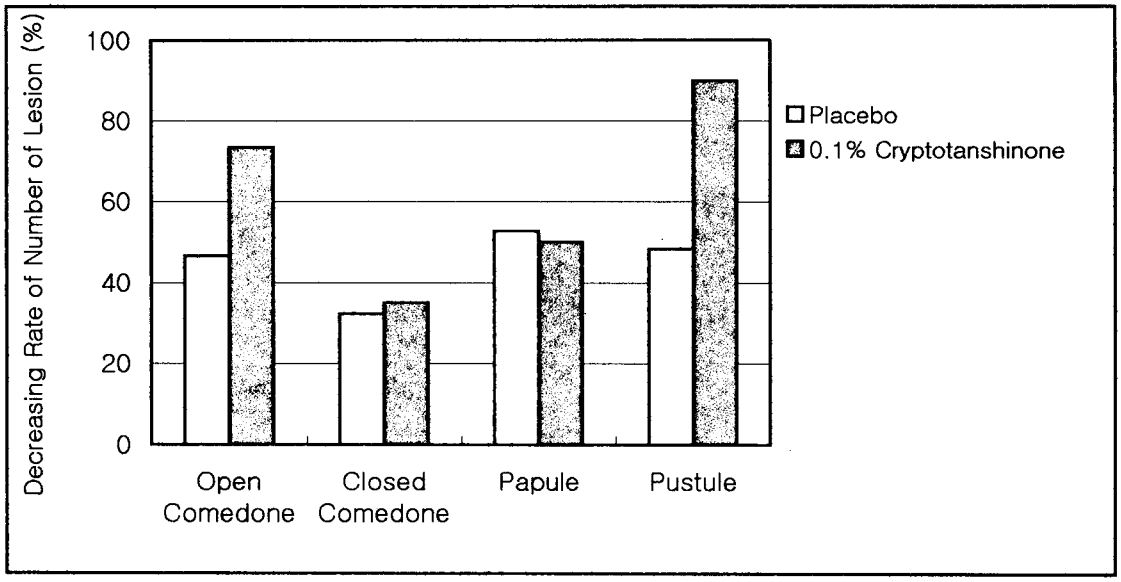

Figure 5. Rate of decrease in the number of lesions after 8 weeks in a clinical study in which skin treatment with an o/w emulsion containing 0.1% cryptotanshinone was compared to treatment with a placebo. Lesions were counted in a 3.5 cm by 2.5 cm area on the cheek. (30 subjects in each group)

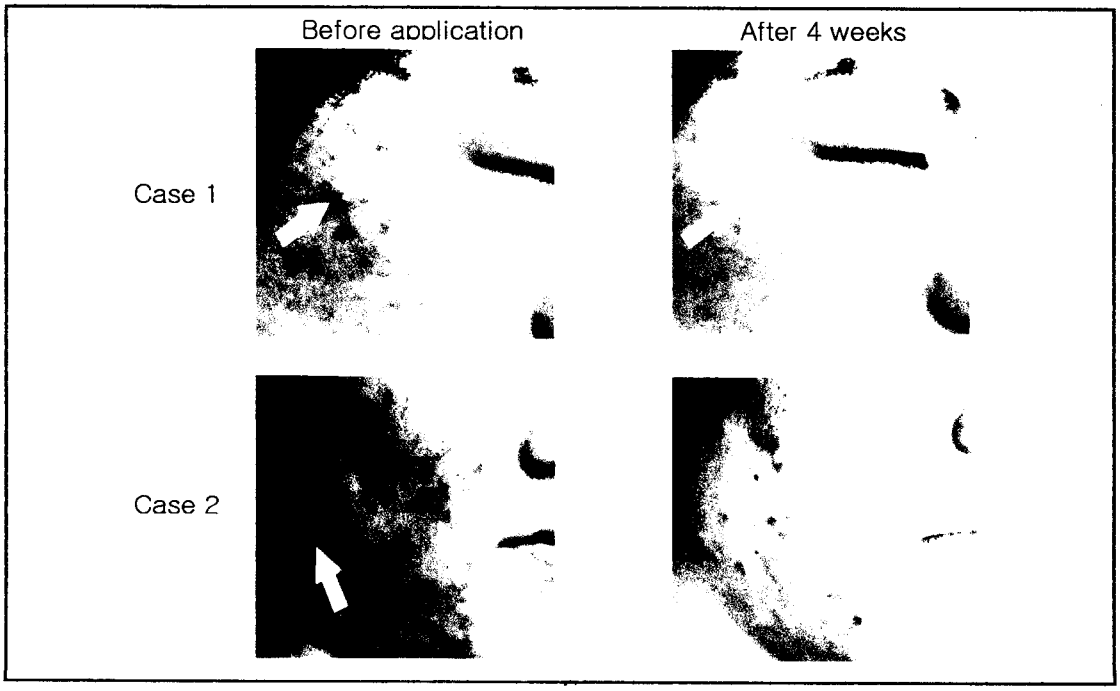

Figure 6. Change in appearance of acne lesions following the application of an o/w emulsion containing 0.1% cryptotanshinone for 8 weeks of treatment. The white arrows indicate corresponding lesion sites.

Because the disruption of comedones initiates an inflammatory reaction, and finally results in pustules[19], cryptotanshinone decreases the number of pustules by its anti-inflammatory activity.

Conclusion

The stability of cryptotanshinone was evaluated at two different temperatures (25□□ and 40□□), at three different pHs(pH 5, 6, 7) and under UV in o/w emulsion. Cryptotanshinone has good stabilities at the tested pHs and very poor stability against UV.

In order to estimate the toxicity of cryptotanshinone, local lymph node assay test for the identification of contact dermatitis and allergic potency and toxicity tests were performed. Cryptotanshinone showed good skin safety.

For estimation of anti-acne effectiveness, an emulsion containing 0.1% cryptotanshinone was applied over a period of eight weeks and the number of acne lesions and clinical measurement of all test participants were determined. The cosmetic composition containing cryptotanshinone can be used safely and effectively without any side effects on skin.

These results suggest that cryptotanshinone would be an effective anti-acne agent for reducing the acne vulgaris. It has demonstrated the ability to manage various grades of acne, from mild to moderate, which include comedones and pustules. Significant improvement is visible within seven days. The high degree of cryptotanshinone's effectiveness lies in its ability to reduce the volume of *P. acnes*. Moreover, the compound also has 5α-reductase inhibition activity leading to a decreased sebum production and anti-inflammatory effects to reduce acne vulgaris. Any side reactions, either long term or short term, were not observed.

Cosmetic compositions containing cryptotanshinone can be formulated to contain additional materials for removing abnormal keratinization, which leads to thickening of the follicular wall in the pilo-sebaceous apparatus. Among these materials are salicylic acid, retinoic acid or retinoic acid derivatives that have a comedolytic effect.

Therefore, we would propose the use of cryptotanshinone as an active ingredient for the prevention and treatment of acne vulgaris lesions, as well as for the management of hyperseborrheic states associated with acne vulgaris.

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