Study of Possible Pharmacological Actions of *Aloe arborescens* MILLER on Mouse, Hamster and Human Skin

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Aloenin is a major constituent of *Aloe arborescens* MILLER which has been utilized in Japan as a folk remedy for burns, insect bites, and skin reaction. In the present study, the effects of aloenin on sebaceous gland size, hair growth and damaged skin were investigated. Aloenin significantly promoted hair growth in depilated mice, but did not affect sebaceous gland function in the hamster ear. Aloenin also had recuperative effects on tape-stripped human skin as determined from parameters such as the shape factor of corneocytes, thick abrasion, nuclear ghosts and cellular arrangement of corneocytes. Since aloenin is effective in healing damaged skin, it may be useful for the treatment of dermatological conditions in the future.

Keywords — *Aloe arborescens* MILLER; aloenin; dermatology; sebaceous gland; hair growth; corneocyte

Introduction

In Japan, *Aloe arborescens* MILLER has been utilized as a folk remedy not only for internal diseases such as constipation and gastrointestinal disorders, but also for surgical applications, burns, insect bites and rough skin.¹⁾ We previously found that several constituents in aloe have inhibitory effects on carrageenan-induced inflammatory edema in the rat paw²⁾ and on histamine release from rat peritoneal mast cells.³⁾ Recently, aloe has also been used in health foods and cosmetics.^{1b)} The constituents of aloes in cosmetics on the market are mainly mucous polysaccharides without barbaloin and aloemodine, called anthranquinone compounds.⁴⁾ Cosmetics containing aloe have been mostly utilized for skin care or to improve the skin

condition.⁵⁾

To determine the usefulness of aloe in dermatology, we examined its effects on the skin with respect to: 1) the effect of aloenin,⁶⁾ a major component of the aloe, on sebaceous gland size in the hamster ear, 2) its stimulative effect on hair growth in mice, and 3) its effect on damaged human skin.

Experimental

Animals — Seven-week-old female Syrian hamsters and 7-week-old male C3H/He mice were purchased from Japan SLC Co., Ltd., Shizuoka, Japan. They were kept in rooms with a controlled temperature ($23 \pm 1^\circ\text{C}$) and humidity ($55 \pm 15\%$) under a 12-h dark-light cycle for one week before the experiments, and also were kept under these conditions during the experiments.

Materials — Dried leaves of *Aloe arbores-*

cens M. were obtained from Aroe Seiyaku Co., Ltd., Shizuoka, Japan. Aloenin was isolated from *Aloe arborescens* M.,²⁾ and identified by comparing physicochemical data (mass spectra, nuclear magnetic resonance and infrared spectra) with data of the standard.

Preparation of Aloe Extract — First, 100 g of dried leaves of *Aloe arborescens* M. was extracted with 1000 ml of methanol by refluxing for 2 h. The extract was filtered and the filtrate was condensed under reduced pressure to yield a brown residue at a yield of 14%.

Test Sample Preparation — Aloenin and aloe extract were dissolved in ethanol (95% v/v) at the designated concentrations before use for each test (control of sebaceous gland secretion and effect on hair growth). Aloenin and control creams were prepared as shown in Table I.

Measurement of Sebaceous Gland Size⁷⁾ — Hamsters were used in groups of five. As shown in Fig. 1, approximately 20 μ l of the test sample was applied to the right ear and ethanol as control to the left ear of each hamster, once daily for 14 d using a swab.

Fig. 1. Structure of Aloenin

Fig. 2. Protocol for Sample Treatment of Ear Skin of Male Hamsters

↓ ; test sample treatment, × ; tissue specimen of ear sebaceous glands taken, ○ ; fixation of ear sebaceous gland specimen and ● ; measurement.

The day after the final application, ear skin biopsies were performed. Each biopsied skin specimen, which was a disc 5 mm in diameter, was fixed in 10% formalin solution for 24 h, stained with Oil Red O, and embedded in glycerin. The stained sheet was placed on a glass slide and used for the measurement. The sebaceous gland area was measured in an image analyzing system (Dimension analyzer, Cosmozone IS, Nikon, Tokyo), composed of a light microscope, projection unit, calculation management unit and input digitizer.⁷⁾ The area was calculated by tracing the periphery of the projected image on the digitizer in a clockwise direction, using the cursor. Six sebaceous gland areas were measured per section, and the mean value for each animal was calculated to obtain a representative value.

Measurement of Hair Growth Promoting Effect⁸⁾ — Four mice per group were used. The back of the mouse was cleared of downy hair by application of depilatory cream (Jully, Kowa Co., Ltd, Nagoya) after cutting the coarse hair under an unanesthetized and unrestrained condition, and a test area of 3.0 × 4.0 cm per mouse was prepared. Beginning the day after depilation, approximately 0.3 ml of test sample was applied to the test area of each mouse once a day using a syringe. One mM 12-*O*-tetradecanoylphorbol 13-acetate (TPA) in ethanol was used as a positive control. Of the three irritant values of color (X, Y and Z) in the test area, only Y was measured by a colorimeter (CR-100 colorimeter, Minolta Co., Ltd., Japan), and was converted to L value⁹⁾ (lightness value of Hunter's Colorimetric System).

TABLE I. Profile of Creams Containing Aloenin

Material	Cream	
	Aloenin	Control
Aloenin	0.5(%)	0(%)
Span-60	1.3	1.3
Tween-60	3.7	3.7
Cetanol	4.0	4.0
Liquid paraffin	30.0	30.0
Methyl paraben	0.2	0.2
Water	60.3	60.8

TABLE II. Standard Score for Evaluation of Corneocytes

Measurement item	Score		
	1	2	3
Thick abrasion	marked	moderate	slight
Nuclear ghosts	many	moderate	few
Cellular arrangement	irregular	moderately irregular	regular
Parakeratotic cells	≥ 5	1—4	0

A 2.4 cm² area was viewed in each corneocyte specimen.

Morphological Evaluation of Skin^{10,11)} —

The inner aspect of the upper arm of 3 men (age = 30, 33 and 35 years) was washed in advance using absorbent cotton soaked in water. Six testing areas were prepared on each man, and an area (15 × 15 mm) was damaged five times using a piece of cellophane adhesive tape (Nichiban Co., Ltd., Japan). About 0.2 g of the test sample was rubbed onto each test site using a finger twice a day, morning and evening, for 30 d, and was also applied after washing following each measurement. The corneocytes were removed from the surface skin at 1, 5, 17 and 30 d after tape-stripping. The surface corneocytes were prepared and the cells transferred from the adhesive tape by a modified method of Kashibuchi.¹⁰⁾

The arrangement of corneocytes, parakeratotic cells, nuclear ghosts and thick abrasion were evaluated by assigning scores for the micrographs according to the standards shown in Table II.¹¹⁾ For morphological evaluation of the cells, the surface area, shape factor, and the perimeter of 10 cells per area selected at random were measured using a microscope equipped with an image analyzing system. The shape factor was calculated from the formula ($4\pi \times \text{area} / (\text{perimeter})$).²⁾

Statistical Analysis — The results, expressed as mean $\pm$ S.E., were statistically analyzed by Student's *t*-test.

Results

1. Effects on Sebaceous Gland Size

As shown in Fig. 3, aloenin had no effect on sebum secretion compared with ethanol as

Fig. 3. Effects of Aloenin and Aloe Extracts on Area of Sebaceous Glands in Ear Skin of Male Hamster

Six sebaceous gland areas per hamster ear were measured by a dimension analyzer. Each column represents mean $\pm$ S.E. obtained from 5 hamsters in one group.

control. However, the aloe extract reduced the size of the sebaceous glands by 12.6%, a significant difference compared with the control ($p < 0.05$).

2. Effects on Hair Growth

Fig. 4 shows the results together with the effect of TPA. The L values of all groups decreased from 9 d after treatment. Aloenin significantly promoted hair growth compared with the control ($p < 0.001$) from 11 d after treatment. The effect of aloenin was similar to that of TPA. Also, hair growth was significantly promoted in the 10% aloe extract group compared to the control group ($p < 0.001$).

3. Effects on Human Skin

Figure 5a—f shows the changes in epidermal structure and the arrangement and morphology of the surface corneocytes after tape-stripping. The aloenin cream group showed a slightly smaller surface area of corneocytes

Fig. 4. Effects of Aloenin and Aloe Extracts on Hair Growth of Mice

L value was measured by a photograph with a colorimeter. Each point represents mean $\pm$ S.E. obtained from 4 mice in one group.

a) $p < 0.01$, b) $p < 0.001$: Significant difference from the control.

than those in the no treatment group and the control group (Fig. 5a). The value of the shape factor of corneocytes in the aloenin cream group was high and stable at all times, and was 0.921 ± 0.004 on day 30 after tape-stripping (Fig. 5b). There were no significant differences in the inhibitory effect on thick abrasion between the aloenin cream group and the other groups to the 17th day. However, aloenin cream significantly inhibited thick abrasion on day 30. In the aloenin cream group, very few corneocytes were peeled off as a thick layer (Fig. 5c).

Also, the inhibitory effect of aloenin cream on nuclear ghosts was similar to that of control cream and no of treatment up to 17 d after tape-stripping. Aloenin cream significantly inhibited nuclear ghosts from day 17 through day 30 (Fig. 5d).

The cellular arrangement score in the aloenin cream group differed from that in the other groups, having no irregularity immedi-

ately after tape-stripping and showing rapid healing. Especially, the cellular arrangement of the corneocytes in the aloenin cream group was significantly more uniform and more regular than that in the other groups on day 30 (Fig. 5e).

There was no significant difference in the inhibitory effect on parakeratotic cells in the corneocyte layer between the aloenin cream group and the other groups (Fig. 5f).

Discussion

Excessive secretion of sebum is known to cause skin disorders such as dermatitis seborrheica, and acne vulgaris, and conversely, insufficient secretion of sebum leads to chapping, rough skin, and dermatitis hiemalis.¹²⁾ Fukushima *et al.* demonstrated that 1% γ -orizanol and 1% cypoterone acetate significantly increased and decreased the size of sebaceous glands, respectively.^{7b)} In this study, 1% aloenin had no effect on sebaceous gland activity of the hamster ear, however, 5% aloe extract reduced the size of the sebaceous glands. These findings suggest that aloe contains constituents other than aloenin, that inhibit sebaceous secretion.

Improving the blood circulation in scalp skin by stimulants and expanders, supplying nutrients to the hair follicles, and administration of female hormone antagonists has been attempted to prevent the loss of hair and improve hair growth.¹³⁾ In this study, 5% aloenin promoted hair growth. However, the effect of aloenin was weaker than that of TPA. Both 5% and 10% aloe extracts also showed a promotive effect. The content of aloenin in the aloe is 11.1–14.9 mg/g,¹⁴⁾ thus the promotive effects of 5% and 10% aloe extracts are believed due to the stimulatory activity of aloenin. Also, we assume that aloenin shows promotive activity at a concentration below 5%.

Kashibuchi^{10,11)} reported a method of exfoliative cytology for morphological evaluation of the skin. Moreover, he found that some

Fig. 5. Effects of Cream Containing Aloenin on Stratum Corneum of Human Skin Damaged by Tape-Stripping

●; aloenin cream, ○; control cream, ■; no treatment.

The surface area (a), shape factor (b), score of thick abrasion (c), nuclear ghosts (d), cellular arrangement (e) and parakeratotic cells (f) were used as measurement items for morphological evaluation of damaged human skin.

Ten corneocytes per testing area were measured with an image analyzing system at random for determining the surface area and shape factor. Each point for the surface area and shape factor represents mean $\pm$ S.E. obtained from 30 determinations.

Thick abrasion, nuclear ghosts, cellular arrangement and parakeratotic cells were evaluated by the micrograph-based scores. Each evaluation was examined three times per testing area. Each point for thick abrasion, nuclear ghosts, cellular arrangement and parakeratotic cells represent the mean score $\pm$ S.E. obtained from 9 determinations.

a) $p < 0.05$, b) $p < 0.01$: Significant difference from the control.

parameters, such as the shape factor of corneocytes the thick abrasion, the nuclear ghosts, and the cellular arrangement of corneocytes, indicate the state of the epidermis, and these findings correlated with subjective evaluation.¹⁰⁾ For example, the diversity of corneocyte size can be recognized by determining their shape factor, revealing many markedly transformed corneocytes in rough skin. In areas of healthy skin, the cells were removed in a monolayer, whereas in areas of rough skin, the cells could be peeled off as a thick layer. Also, since the cellular arrangement of corneocytes in the morphological evaluation of skin correlates with the roughness to the touch on subjective evaluation of skin, this cellular arrangement is an important parameter in skin evaluation, as is the

shape factor of corneocytes. The surface corneocytes of healthy skin form a regularly arranged sheet.

In this study, aloenin had effects on the shape factor of corneocytes, thick abrasion, nuclear ghosts, and corneocyte cellular arrangement. These findings suggest the effectiveness of *Aloe arborescens* M. in the treatment of rough skin, and also in the maintenance or improvement of skin surface corneocytes.

They also show that aloenin, a major constituent of *Aloe arborescens* M., is effective for improving rough human skin. The use of aloe as a folk remedy is thus supported by the present study. Furthermore, aloenin seems to be a valuable constituent of aloe and should be examined in future dermatological studies.

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