Biobenefity®

(Cynara Scolymus (Artichoke) Leaf Extract)

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NF-κB and Photo-Aging[1) to 7)]

Human body has the genetic information of about twenty-two thousand genes and has been maintained by the information. The gene exhibits its function by expressing the appropriate amount of information where and when the information is needed. When the information is not necessary, the gene is locked so as not to exhibit its function. The key to unlock the gene is called "Transcriptional factor." One of the representative transcriptional factors is "Nuclear factor-kappa B <NF-κB>."

NF-κB is activated in response to various stimulus events to increase the expression of various genes involved in immunity, proliferation, apotosis, inflammation and so on (Fig. 1). In

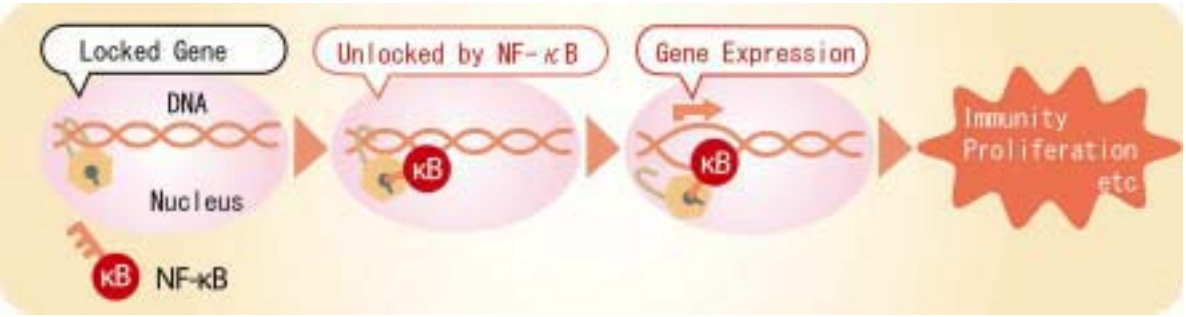


Fig.1 Expression of various genes by NF-κB

a normal condition, NF-κB unlocks a gene only when the gene expression is necessary. When a cell is led to an abnormal condition by inflammation etc., NF-κB is activated enormously to excessively enhance the gene expression (Fig. 2). It has been considered that such excessive gene expression induces various disorders. In fact, it has been reported that excess activation of NF-κB leads to the occurrence of cancer, rheumatism, psoriasis and so on. Therefore, NF-κB inhibitors are now under development extensively in the world.

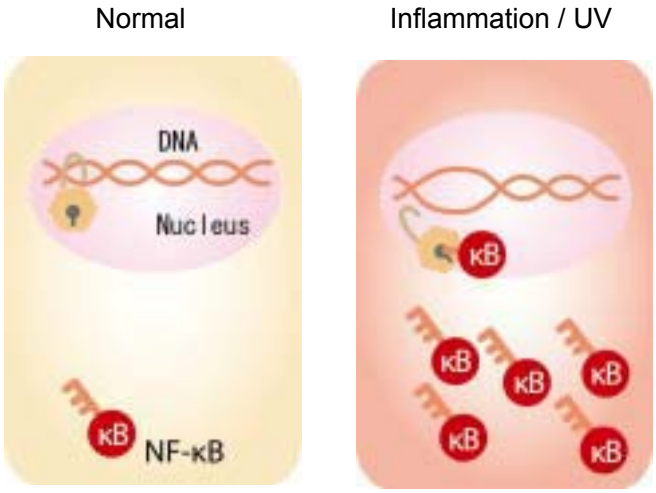


Fig.2 Inflammation and Excessive NF-κB

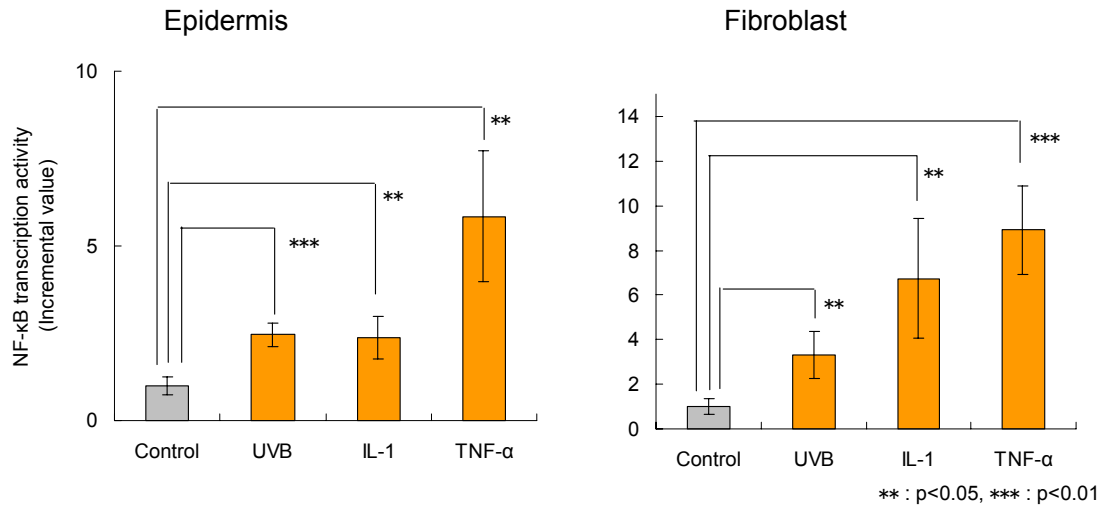


Fig.3 NF- κ B Transcriptional Activity due to the variety of Stimulation

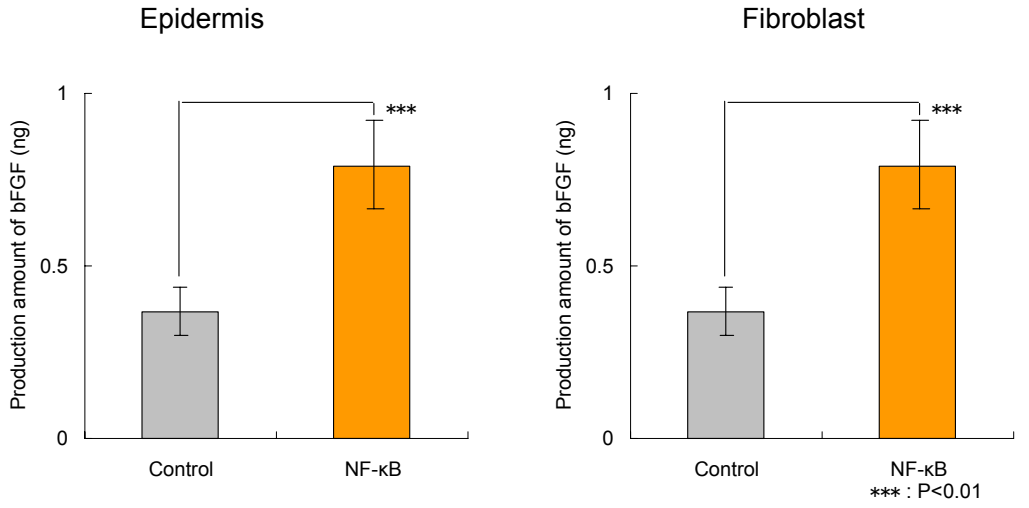


Fig.4 bFGF and MMP-1 Production Effect by NF- κ B

NF- κ B exists also in skin epidermal keratinocyte and dermal fibroblasts, and it has been confirmed that NF- κ B is expressed excessively by stimuli such as ultraviolet radiation and inflammation to result in an increase in the transcriptional activity (Fig.3). Excess expression of NF- κ B induces inflammatory cytokines in the cells which further induces the expression of NF- κ B. That is, uncontrollable spiral production of NF- κ B occurs. Excess expression of NF- κ B increases the production of bFGF (basic Fibroblast Growth Factor), a cell growth factor (Fig. 4), which causes epidermal thickening due to hyperproliferation and abnormal keratinization of epidermal keratinocytes and pigment deposition due to melanocyte proliferation. It is considered that such

changes lead to photo-aging of the skin (Fig. 5).

We confirmed that NF-κB played a major role in photo-aging, verified that various photo-aging symptoms could be prevented by NF-κB inhibitors (Fig. 6) and published the results *. We have confirmed at the same time that the inhibition of the NF-κB action does not give adverse effects on normal skin functions [7].

It can be expected that NF-κB inhibitors have the capability to safely protect the skin from photo-aging.

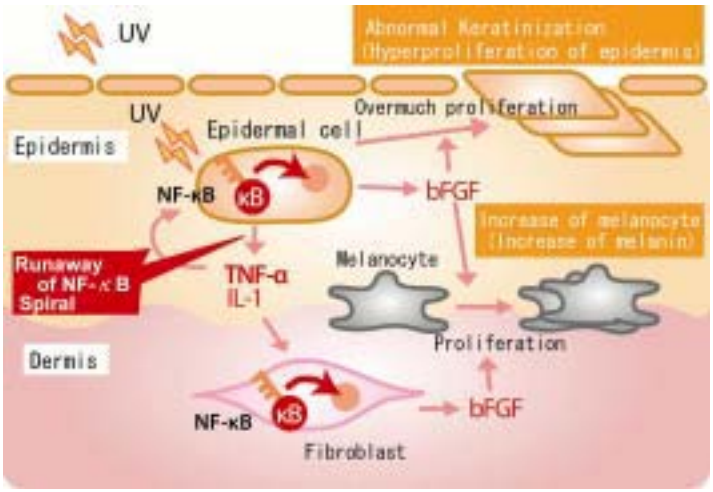


Fig.5 Photo aging and NF-κB

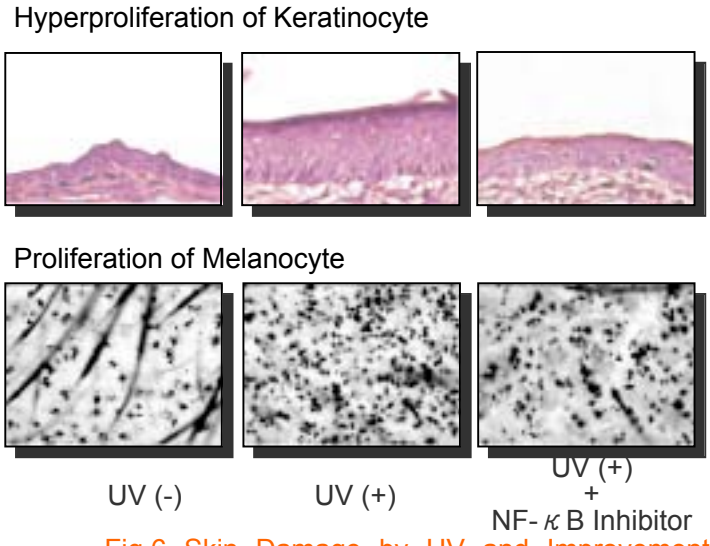
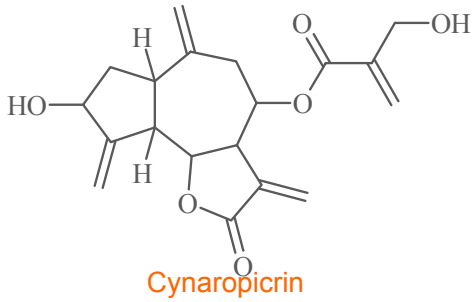


Fig.6 Skin Damage by UV and Improvement Effect of NF-κB

*124th Japan Pharmaceutical Society meeting (2004)
104th meeting of Japanese Dermatological Society (2005)
J.Pharmacol. Exp. Ther (2005)

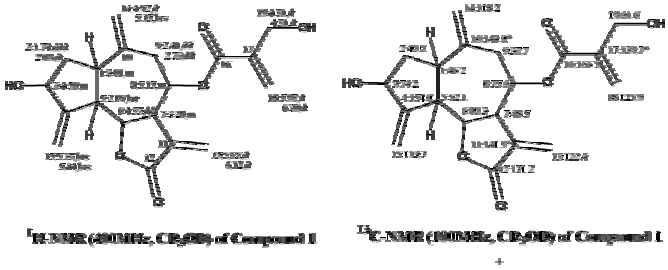
Cynaropicrin and Biobenefity[7) 8)]

Since it can be expected that NF-κB inhibitors have the capability to protect the skin from photo-aging, a screening test of the candidates was performed on the basis of the inhibitory activity on the increment of NF-κB gene transcription stimulated by TNF-α (Tumor Necrosis Factor-α) in a cell. In the screening test, we found that artichoke extract had a powerful inhibitory activity on the increment of NF-κB transcription. Thus,



Cynaropicrin

$C_{19}H_{22}O_6$ MW=346



artichoke extract was purified by silica gel column chromatography etc. NMR (H, C) analysis of an isolated component revealed that an active component of artichoke extract was "cynaropicrin" (Figure shown below). It was cleared that cynaropicrin had very strong inhibitory activity on TNF-α dependent enhancement of NF-κB transcription in a concentration-dependent manner (Fig.7. presented at the 125th Annual Meeting of the Pharmaceutical Society of Japan).

Thus we developed "Biobenefity", highly purified artichoke extract, prepared by removing almost all components except cynaropicrin from artichoke crude extract using various column chromatography purification methods.

Since Biobenefity contains cynaropicrin with powerful inhibitory action on an increase in NF-κB transcription, it can be expected to have a powerful capability to protect the skin from photo-aging. The comparison of a capability to inhibit an increase in NF-κB transcription between Biobenefity and cynaropicrin alone revealed that both compounds had almost the same capability on the basis of cynaropicrin unit (Fig. 8). Therefore, it was cleared that the powerful effect of cynaropicrin was exhibited also in Biobenefity.

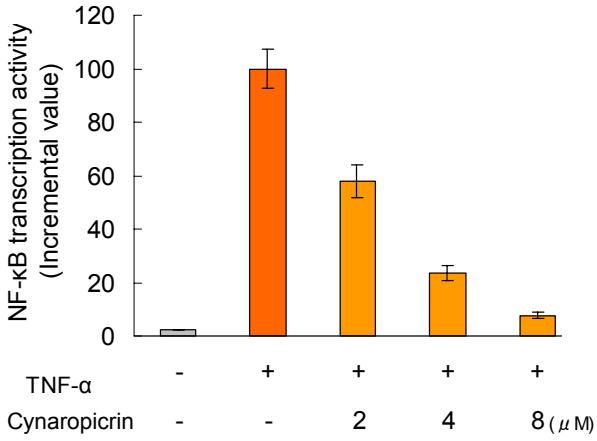


Fig.7 Inhibitory Effect of NF-κB Transcriptional Activity on Cynaropicrin

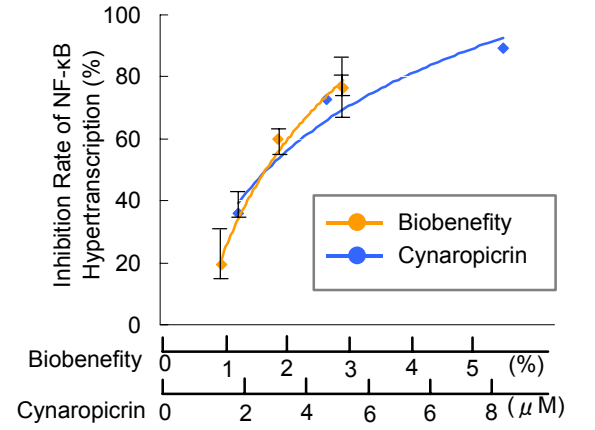


Fig.8 Comparison between Cynaropicrin and Biobenefity

Cynara scolymus L. (*Compositae*)[9) to 12)]

Biobenefity is extract obtained from the above ground portion of *Cynara scolymus* L. (*Compositae*), genus Cynara in Composite family. Artichoke is called artichoke in English, Artichaut in French and Chosenazami in Japanese. It has been said that the original home of Cynara scolymus L. is European Mediterranean coastal region and African Northern region. It has been, however, considered that Cynara scolymus L. was produced by improving *Cynara cardunculus* originally grown wild in Mediterranean Midwest region and used the leafstalk for food. Cynara scolymus L. was cultivated in Italy in the 15th century and it was enthusiastically improved much better for food vegetable in France from the 16th century and afterwards. It came to Japan from the Meiji era and the majority of the plant commercially available now has been imported from EU or American continent.

Cynara scolymus L. is 60 to 200 cm in height, the leaves have long deeply serrated bases and thick white tomenta on the back. A large purple head-like inflorescence with about 15 cm in diameter blooms early summer to summer. The pulpy portion of involucral bracts of buds just before bloom is used as herbal medicine and as food after boiling. The flower is enjoyed as cut flowers.

The tori and involucres contain cynarin, chrologenic acid, caffeic acid, protein, carotene, vitamin C etc. and it has been known that the leaves, tori and involures have various pharmacological actions such as diuresis, making the body strong, acceleration of bile secretion, a decrease in blood cholesterol concentration and liver protection. Therefore, they have been used for the treatment of arteriosclerosis, jaundice and dyspepsia, and appetite enhancement.

Biobenefity is highly purified extract of artichoke prepared by removing almost all components except cynaropicrin, an active component, from crude extract of artichoke with various column chromatographies. By such high purification, the product has very good stability and compatibility.

Biobenefity was named using bio and benefit, which means that it is a raw material for cosmetics having benefit of bio (plant) cultivated on the earth.

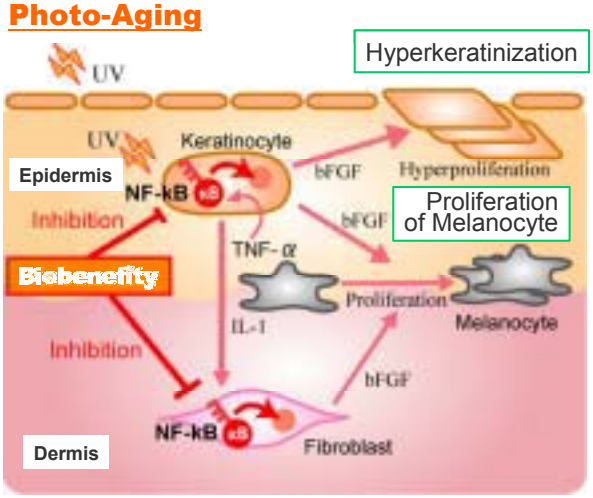
Introduction

Biobenefity is a 1,3-butylene glycol water solution to dissolve an ethanol extract of an aerial part of *Cynara scolymus* L. (*Compositae*).

Efficacy

Biobenefity inhibits NF-κB Hypertranscription in skin and below mentioned prevention of photo-ageing was observed.

Photo-Aging



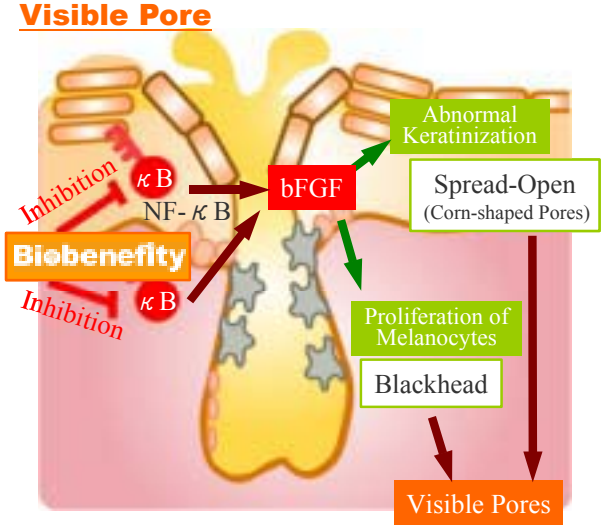
in vitro

- Inhibitory Effect of bFGF Production
- Inhibitory Effect of Epidermal Melanocyte Proliferation
- Inhibitory Effect of B16 Melanoma Cell Production

in vivo

- Whitening Effect on Human Skin
- Improvement Effect of Skin Elasticity on Human Skin

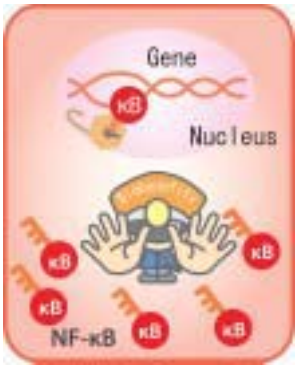
Visible Pore



Also, prominent skin's pores was observed by Inhibitory Effect of NF-κB Hypertranscription

in vivo

- Improvement Effect of Pore on Human Skin



Biobenefity ("Anti-aging·Security" Raw Material); which protect from excessive NF-κB

Inhibitory Effect of NF-κB Hypertranscription

Transcription of NF-κB gene is enhanced by stimulus such as inflammation and ultraviolet irradiation in the skin cells to lead to epidermal hyperkeratinization and disrupture of extracellular matrix such as collagen, which causes photo-aging of the skin.

The effect of Biobenefity on TNF-α dependent NF-κB transcription was examined in human cells.

Test Sample

Biobenefity is applied 1 to 3% as final concentration. As control, 50% 1,3-Butylene Glycol was applied.

Test Method

293 cells (human embryonic kidney cells, Riken) were seeded on a 12-well dish using DMEM (Sigma) containing 10% fetal bovine serum (Thermo Trace). At 24 hours after seeding, firely luciferase reporter vector, pGL-3-BasicVector with a base sequence of NF-κB binding site and an internal standard, pRL-TK Vector expressing renilla luciferase were transfected into the cells with Fugene-6 transfection reagent (Roche Diagnostics). Twenty-four hours later, a test sample was added and 2 hours later, TNF-α was added to make the final concentration 0.5 ng/mL. Twenty-two hours later, cell contents were extracted to assay luciferase activity in the extract with Luciferase Assay System (Promega) by a luminometer (Promega TD-20/20 Luminometer). The amount of NF-κB expressed was obtained by dividing the assay value of firefly luciferase (amount of NF-κB transcribed) by the assay value of renilla luciferase (amount of cells). The amount of NF-κB expressed in the addition of TNF-α was defined as 100 to calculate the relative value in the treatment of each test sample.

Result and Discussion

The effect of Biobenefity on TNF-α dependent NF-κB transcription was shown in Fig.9. Although amount of NF-κB Hypertranscription was caused by stimulation of NF-α, NF-κB Hypertranscription was strongly inhibited by concentration of Biobenefity.

According to this result, Biobenefity is expected to inhibit photo-aging by inhibitory effect of NF-κB hypertranscription.

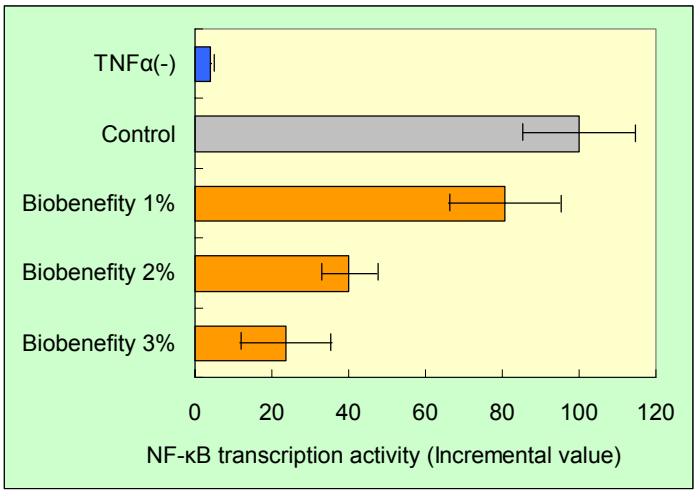


Fig.9 Inhibitory Effect of NF-κB Hypertranscription

Inhibitory Effect of bFGF Production (NF-κB)

bFGF (basic Fibroblast Growth Factor) is produced excessively at the inflammatory sites and the skin exposed ultraviolet rays to enhance abnormal proliferation (hyperkeratinization) of the hyperkeratinization, which leads to dry skin due to the thickness of the horny layer and abnormal differentiation of epidermal keratinocyte, and to pigment deposition. It has been known that NF-κB is expressed excessively at the occurrence of inflammation to increase bFGF production. Therefore, a substance that inhibits NF-κB dependent bFGF production can be expected to prevent horny layer thickness, dry skin and pigment deposition due to inflammation and ultraviolet irradiation (photo-aging).

Using epidermal keratinocyte expressing NF-κB excessively, the inhibitory effect of Biobenefity on bFGF production was examined.

Test Sample

Biobenefity is applied 3% as final concentration. Also, cynaropicrin of active component was applied as same concentration as Biobenefity. As control, 50% 1,3-Butylene Glycol was applied.

Test Method

Normal human epidermal keratinocyte (Kurabo NHEK) was seeded on a 12-well plate (Corning) with serum-free medium EpiLife-KG2 (Kurabo) and preincubated for 24 hours at 37°C under 5% CO_2 condition. Thereafter, pCMV-p65 Vector expressing p65, a subunit of NF-κB, was transfected into the cells with Fugene-6 transfection reagent (Roche Diagnostics) so that the cells are changed to those that can express active NF-κB strongly. After further 24-hour incubation, the medium was changed to fresh one. Then a test sample was added and the cells were further incubated for 48 hours. After the incubation, the supernatant of the culture medium was isolated to determine bFGF with bFGF EIA kit (Cytimmune). At the same time, the number of cells was counted with Cell Counting Kit-8 (Dojindo) to calculate the amount of bFGF per cell, which was used for the evaluation.

Result and Discussion

The inhibitory effect of Biobenefity on bFGF production is shown in Fig. 10. In the modified cells with strong expression of NF-κB, the amount of bFGF produced was significantly increased. Biobenefity inhibited bFGF production significantly in the cells. The addition of cynaropicrin also inhibited bFGF production like the addition of Biobenefity, confirming that cynaropicrin played a major role in the inhibitory effect of Biobenefity.

It was confirmed by above-mentioned results that Biobenefity inhibited bFGF production caused by excess NF-κB. Therefore, it can be expected that Biobenefity improves the aging skin symptoms such as abnormal keratinization and pigment deposition due to inflammation and ultraviolet irradiation.

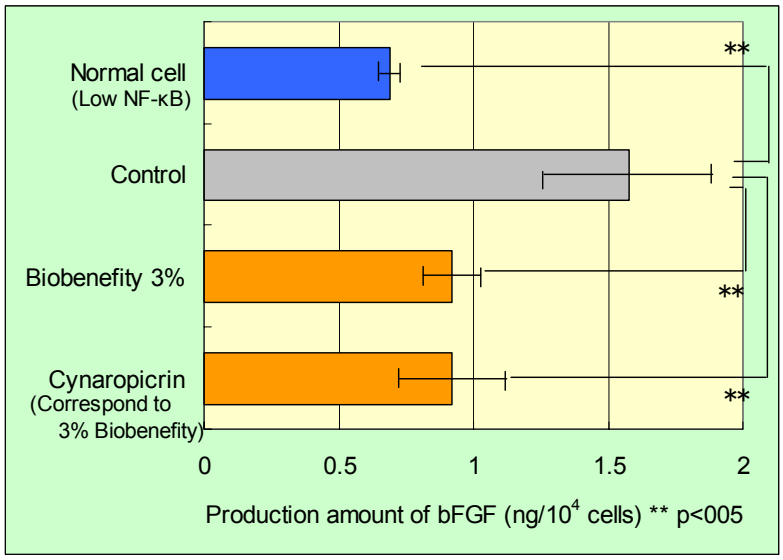


Fig.10 Inhibitory Effect of bFGF Production

Inhibitory Effect of Epidermal Melanocyte Proliferation (NF-κB)

Epidermal melanocyte producing melanin pigment in the skin is increased by ultraviolet irradiation, chronic inflammation, etc. and it has been said that the increased epidermal melanocyte leads to an increase in the amount of melanin production in the skin. NF-κB is expressed excessively in epidermal keratinocyte at the occurrence of inflammation and increases the production of bFGF with proliferating activity on melanin cells in epidermal keratinocyte. Therefore, it can be expected that a substance inhibiting NF-κB activity prevents the occurrence of chloasma and freckle caused by inflammation and ultraviolet irradiation, that is, pigment deposition.

Using human epidermal keratinocyte modified to express NF-κB excessively and human epidermal melanocyte, the inhibitory effect of Biobenefity on epidermal melanocyte proliferation was examined.

Test Sample

Biobenefity is applied 3% as final concentration. Also, cynaropicrin of active component was applied as same concentration as Biobenefity. As control, 50% 1,3-Butylene Glycol was applied.

Test Method

Normal human epidermal keratinocyte (Kurabo NHEK) was seeded on a 12-well plate (Corning) with serum-free medium EpiLife-KG2 (Kurabo) and preincubated for 24 hours at 37°C under 5% CO_2 condition. Thereafter, pCMV-p65 Vector expressing p65, a subunit of NF-κB, was transfected into the cells with Fugene-6 transfection reagent (Roche Diagnostics) so that the cells are changed to those that can express active NF-κB strongly. After further 24-hour incubation, the medium was changed to fresh one. Then a test sample was added and the cells were further incubated for 48 hours and the supernatant of the culture medium was isolated.

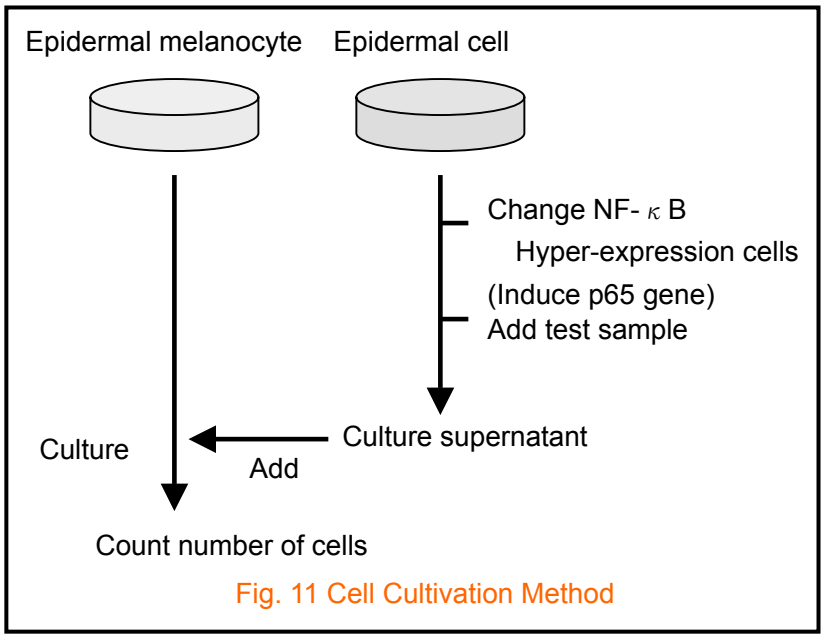


Fig. 11 Cell Cultivation Method

Human epidermal melanocyte (Kurabo NHEM) was seeded on a 24-well plate (Corning) with low-serum medium Medium 154S (Kurabo) and incubated for 24 hours at 37°C under 5% CO_2 condition. Thereafter, the medium was changed to a medium prepared by mixing Medium 154S without a proliferation promoter and the supernatant of epidermal keratinocyte culture medium collected in the previous test at the volumetric ratio of 1 to 4 and the cells were incubated for a further 72 hours (Fig. 11). After the completion of incubation, the cells were isolated and the number of cells was counted with a blood cell counter.

Result and Discussion

The inhibitory effect of Biobenefity on epidermal melanocyte proliferation is shown in Figs. 12 and 13. The addition of the culture medium supernatant of epidermal keratinocyte with excessive expression of NF-κB to the medium for the cultivation of epidermal melanocyte significantly increased epidermal melanocyte proliferation. On the other hand, the culture medium supernatant obtained from epidermal keratinocyte incubated in the addition of Biobenefity did not increase epidermal melanocyte proliferation. In the addition of cynaropicrin, the inhibition of epidermal melanocyte proliferation was also observed like in the addition of Biobenefity. Thus it could be confirmed that cynaropicrin also played a major role in the effect of Biobenefity.

It was confirmed by above-mentioned results that Biobenefity inhibited epidermal melanocyte proliferation caused by excess NF-κB. Therefore, it can be expected that Biobenefity improves the pigmentation such as black spot and freckle to inflammation and ultraviolet irradiation.

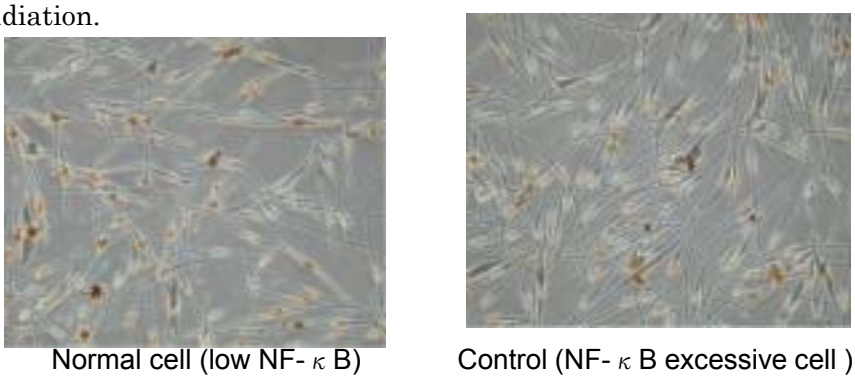


Fig.12 Increase effect of Epidermal melanin cells 1

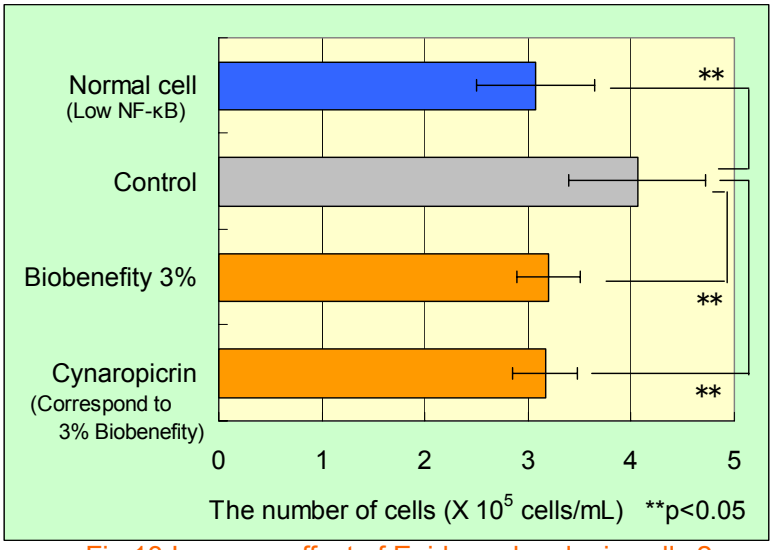


Fig.13 Increase effect of Epidermal melanin cells 2

Whitening Effect on Human Skin

NF- κ B is hyper-released by inflammation in epidermal cell and production of bFGF; which has efficacy of melanin production from epidermal keratinocyte causes hyperkeratinization. We investigate the whitening effect of Biobenefity on human skin.

Test Sample

Biobenefity is diluted 20 times by 50% 1,3-Butylene Glycol, adjusted to 5% solution and use as test sample. 50% 1,3-Butylene Glycol is used as control.

Test Method

The test detail is explained to volunteers in advance, and after confirming consent, they cooperated in these tests. Test sample and control was applied on both cheeks twice a day for 3 months. Volunteers were allowed to use their general cosmetic items during this test. The test was conducted from December 20, 2004 to March 22, 2006

Black (dark) value was measured before application, after application and one to three months later by MEXAMETER MXI8 (Courage + Khazka electronic GmbH, Germany).

Result and Discussion

Transition of melanin index of 5 volunteers is shown in Fig. 14. Melanin index was down on skin applied with Biobenefity improved.

According to this result, Biobenefity has a whitening effect on human.

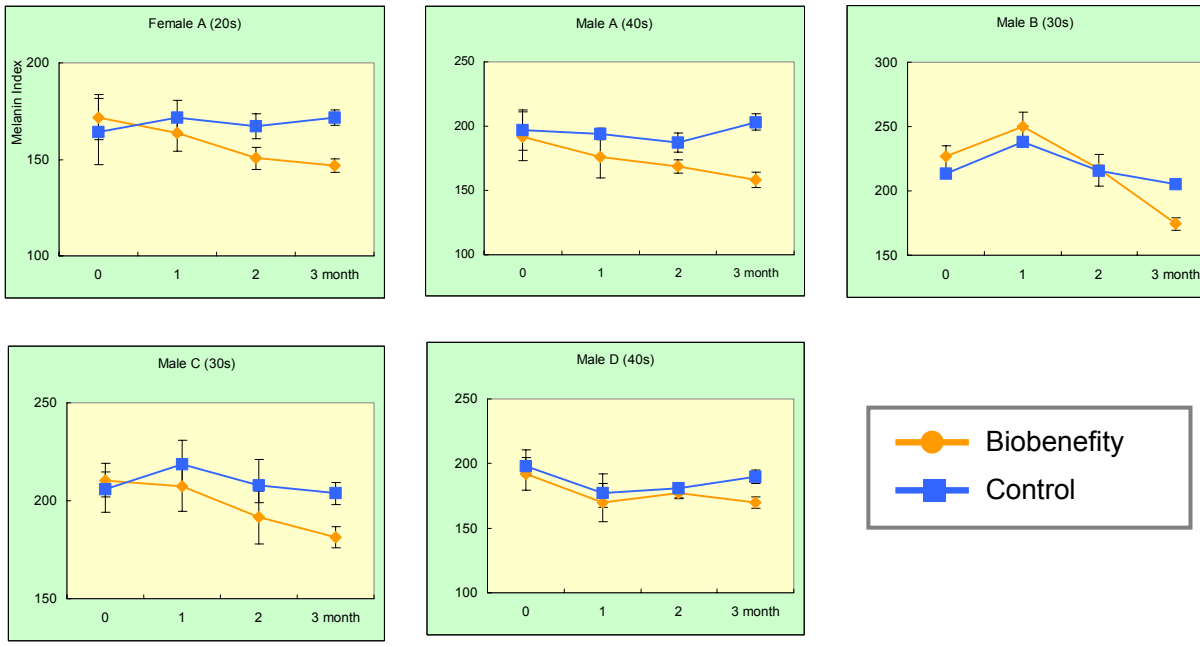


Fig.14 Whitening Effect on Human Skin

Inhibitory Effect of B16 Melanoma Cell

We investigated melanin production inhibition test on B16 melanoma cells for Biobenefity

Test Sample

Biobenefity is applied 1% and 2% as final concentration. As control, 50% 1,3-Butylene Glycol was applied.

Test Method

B16 melanoma cells were used. MEM culture medium (GIBCO BRL) including 5% fetal bovine serum (ThermoTrace) were used and cultured at 37℃ and 5% CO_2. 2 x 10^5 B16 melanoma cells were planted in a 60 mm plastic culture dish and were pre-cultured 24 hours. Then they were transferred to a fresh culture medium and test materials were added. The cells were collected by processing with trypsin after culturing for three days. Cells were dissolved in 1N NaOH and 10% DMSO and then absorbance was measured at 420nm. At the same time, the number of the cells were measured, and amount of melanin per cell were measured and analyzed.

Result and Discussion

Amount of melanin in medium was shown in Fig. 15. Biobenefity was observed to have significant inhibition effect of melanin production.

According to results, Biobenefity is expected to have strong whitening effect.

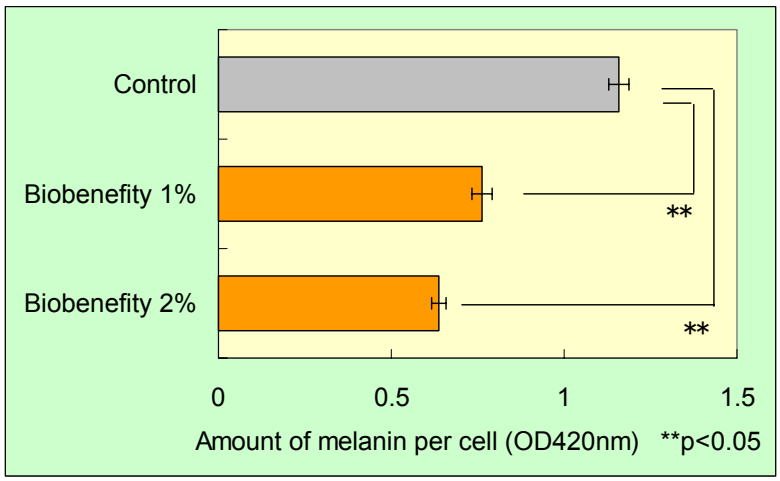


Fig.15 Inhibitory Effect of Melanin

Improvement Effect of Skin Elasticity on Human Skin

A decrease in skin elasticity finally leads to aging symptoms such as wrinkles and pouches. It has been considered that such decrease in skin elasticity is caused by the degradation and denaturation of dermic components such as collagen due to the action of active oxygen and due to inflammation. A decrease in elasticity due to epidermal hardness can be also considered to be one of the causes. Since bFGF produced by the action of NF-κB increases epidermal thickness, there is a possibility that bFGF decreases skin elasticity by hardening the skin. Thus an improving effect of Biobenefity with an inhibitory action on NF-κB on skin elasticity was examined.

Test Sample

Biobenefity is diluted 20 times by 50% 1,3-Butylene Glycol, adjusted to 5% solution and use as test sample. 50% 1,3-Butylene Glycol is used as control.

Test Method[13)]

5 volunteers at age 20s to 40s that gave us written informed consent were enrolled in this study. Each test sample was applied around the left and right eyes of each subject twice a day for 3 months. Volunteers were allowed to use their general cosmetic items during this test. The test was conducted from December 20, 2004 to March 22, 2006.

Before and at 3 months after the commencement of the application, the elasticity of the skin was measured by a skin viscosity and elasticity meter · Cutometer (CUTOMETER SEM474, COURAGE + KHAZAKA Electronic GmbH). The elasticity was calculated by the change of the skin condition when the skin was sucked for 5 sec by instantly reducing the pressure to 500 mb and thereafter the negative pressure was instantly released, which were done twice.

The test was performed several times each in right and left application sites, and the percentage of the change of the mean values between the value before the commencement of the application and that at 3 months after the commencement of the application was calculated to obtain the rate of the change (Ur/Uf). The measurement was performed 20 min after acclimation in an air conditioning room (room temperature: 20°C and humidity: 50%) after washing the face.

Result and Discussion

The change of elasticity before application and 3 months later of 5 volunteers is shown in Fig.16. According to application of Biobenefity, elasticity were improved for 5 volunteers compared with control.

According to this result, Biobenefity has improvement effect of elasticity.

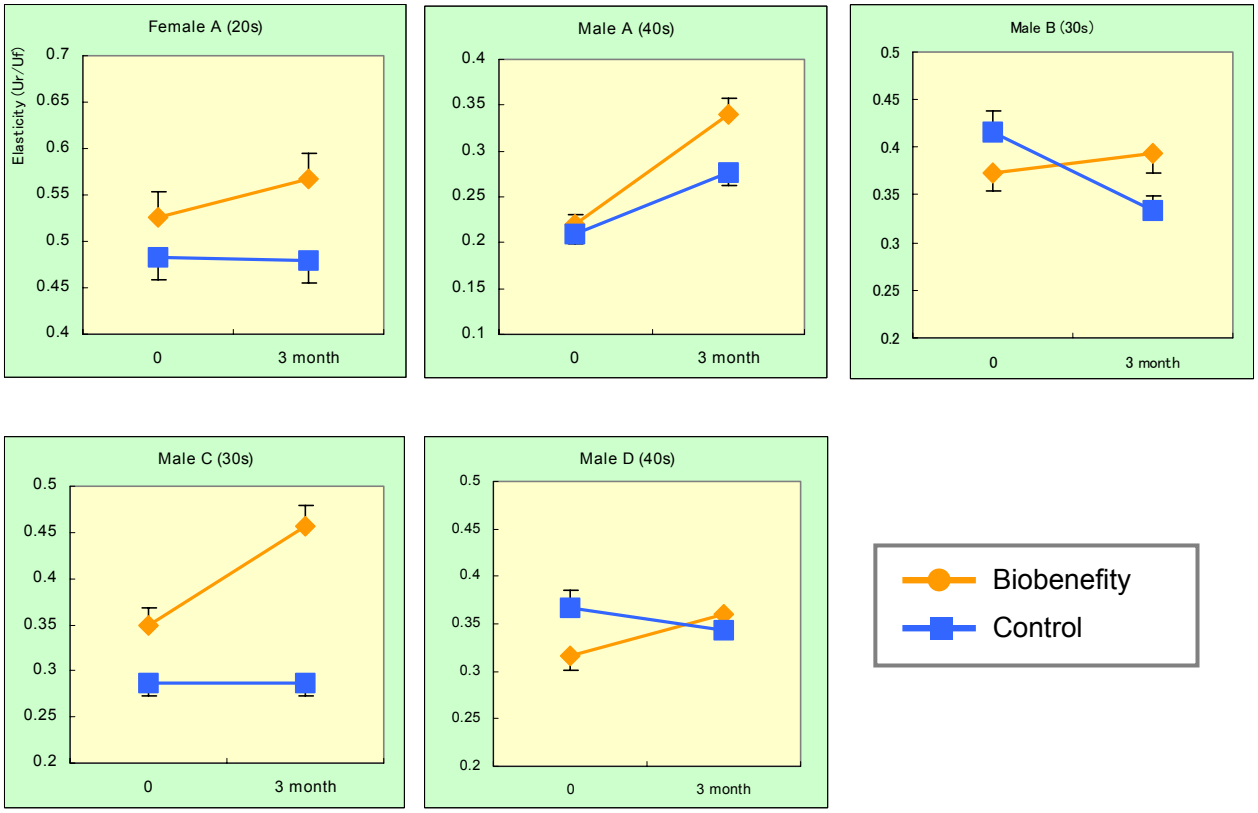


Fig.16 Improvement Effect of Skin Elasticity

NF-κB and Action of Skin Pore[14 to 17)]

One of the major recent skin trouble topics is "Noticease skin pores." Until now, so much attention has not been paid to such topic. The recent beauty skin boom and increased consciousness about the skin appearance because of the increased chance of seeing the skin condition much more closely due to the advent of high resolution digital cameras and high vision are one of the reasons why the concern for skin's pores was heightened.

It is considered that there are roughly two factors for the noticease skin pores (right figure). One is opening of the skin's pores. It has been said that excessive sebaceous secretion makes the skin's pores around the nose open. However, it has been recently reported that the extend of the skin's pores by conically sinking of the pores caused by abnormal keratinization, enhances parakeratosis, in epidermal cells around the skin's pores led to the prominence of the skin's pores.

Another one is darkening of skin's pores. The pores apparently darken due to the dirty staining of sebum of keratotic plug packed in the skin's pores and of keratin. Furthermore, pigment deposition due to ultraviolet irradiation is listed as one of the causes. Epidermal melanocyte exists also in the inside of the skin's pores so that melanin is made in the pores. Epidermis in the pores appears dense from the outside because of the deep positioning. By ultraviolet irradiation, not only dirtiness but also the skin's pores themselves apparently darken.

Spread-Open

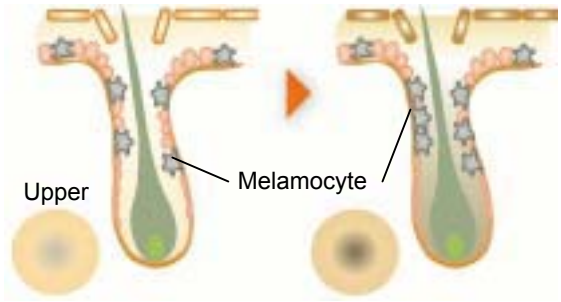
Firm pore

Opened pore by blocking excessive lipid, horny substance etc.

Firmed pore

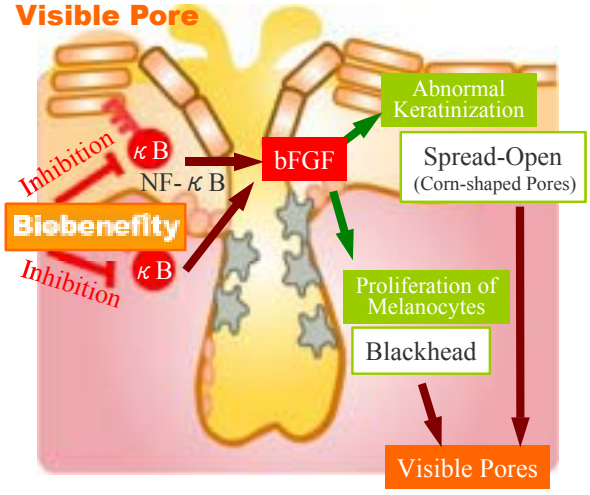
By abnormal keratinization of pore outskirts, a pore opens cone-shaped.

Blackhead



Invisible pore

Pigment cell and melanin are increased. Deep pore, especially, looks black.



It is expected that excess expression of NF-κB may be involved in these two causes for the prominence of the skin's pores (following table). Abnormal keratinization considered as a cause for opening of the skin's pores and an increase in the melanin production causing darkening of pores are phenomenon caused by the increased NF-κB action.

Improvement Effect of Pore on Human Skin

We investigated improvement effect of pore on Human Skin for Biobenefity.

Test Sample

Biobenefity is diluted by purified water, adjusted to 5% solution and use as test sample. 50% 1,3-Butylene Glycol is used as control.

Test Method

Fifteen male and female healthy volunteers in their twenties to forties that gave us written informed consent were employed as subjects in the present study. Each test substance was applied overall on left and right face twice a day for 2 months after face washing. There was no limitation on the usage of cosmetics other than the test substance. The test was performed during June 9, 2005 and August 16, 2005.

Before the commencement of application, and 1 and 2 months after the commencement application, the number of the prominent skin's pores around the nose and the cheek was counted by a Roboskin Analyzer RSA-50 (Inforward, right photo).

The measurement was performed after 20-min acclimation in a constant temperature and humidity controlled room (temperature: 20°C and humidity: 50%) after face washing.

Result and Discussion

The images of the skin's pores before the commencement of application and the representative improvement images of the skin's pores 2 months after the commencement of application at each application site of 15 subjects are shown in Fig. 17 and Fig. 19, respectively, and the change of the number of the skin's pores opened is shown in Fig. 20. Nine of 15 subjects showed a decrease in the number of the skin's pores opened at the Biobenefity application site and three other subjects showed a tendency towards improvement. It was only three subjects that showed no improvement (Fig. 18). There was no subject whose symptom was aggravated compared with the pre-application condition.

From these results, it can be expected that Biobenefity shows an improving effect on the prominent skin's pores.

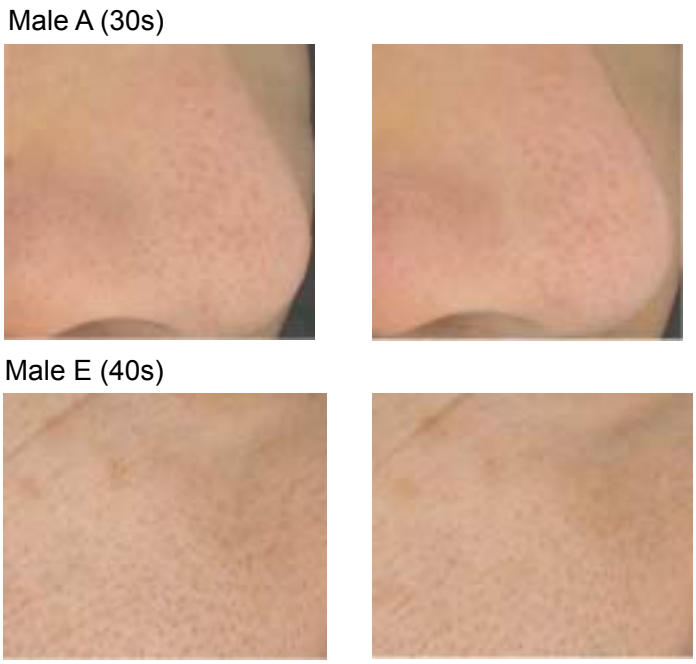


Fig.17 Example of improvement effect of pore

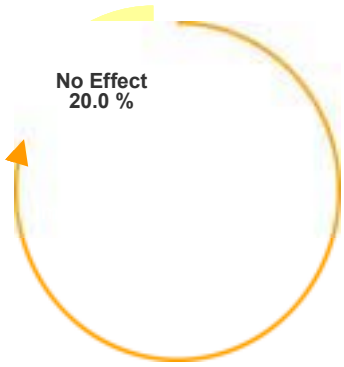
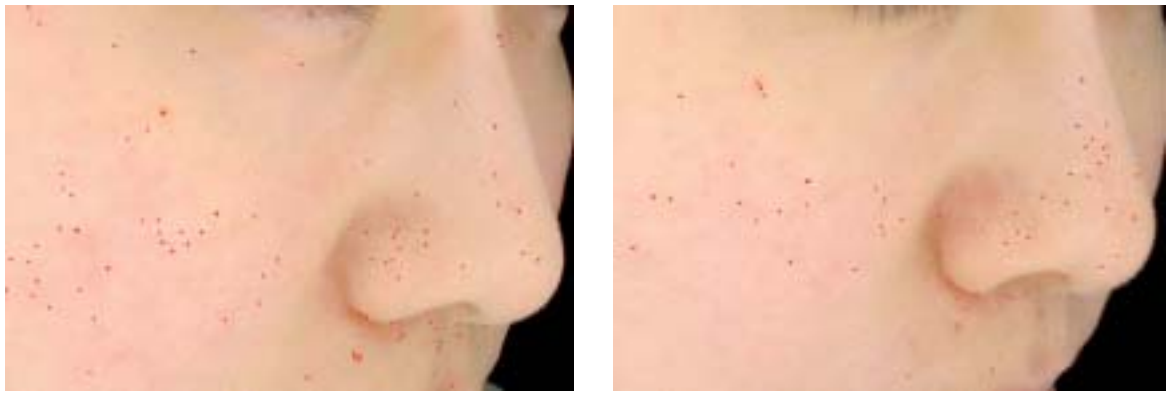
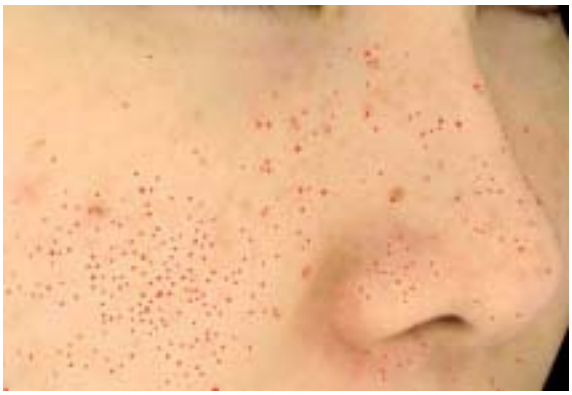


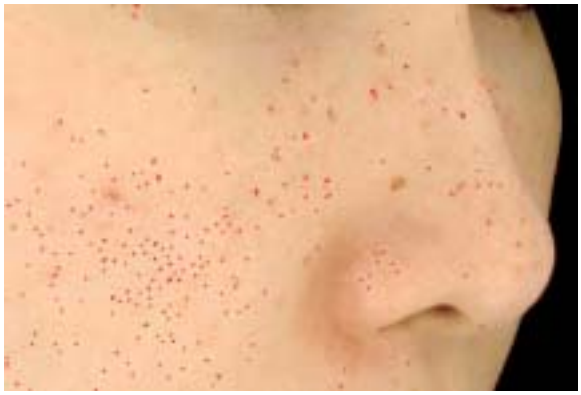
Fig.18 Improvement of skin pore



Female B (20s)

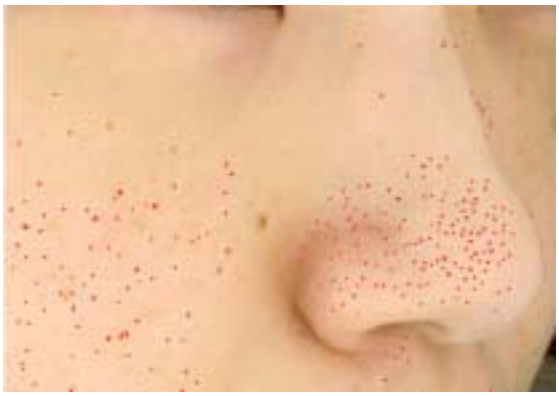


Before Application

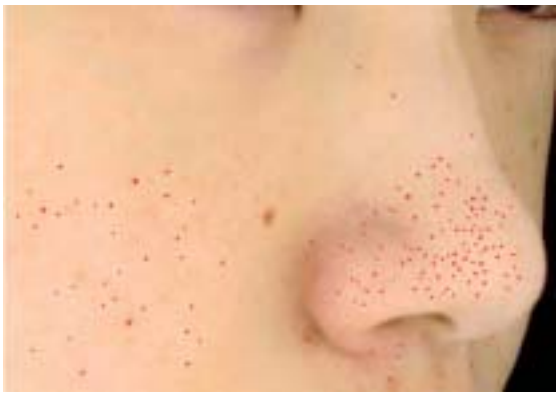


2 Month Later

Female C (20s)

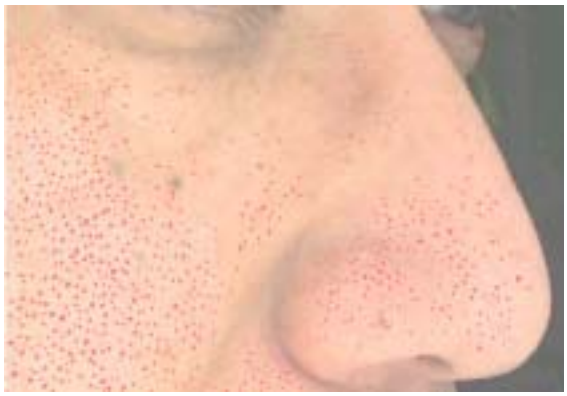


Before Application

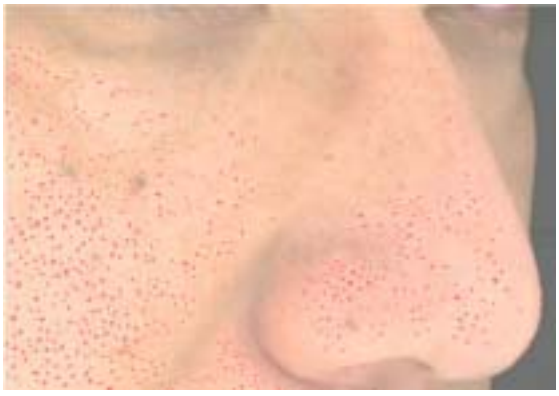


2 Month Later

Male B (40s)



Before Application



2 Month Later

Fig.19 Example of improvement effect of pore

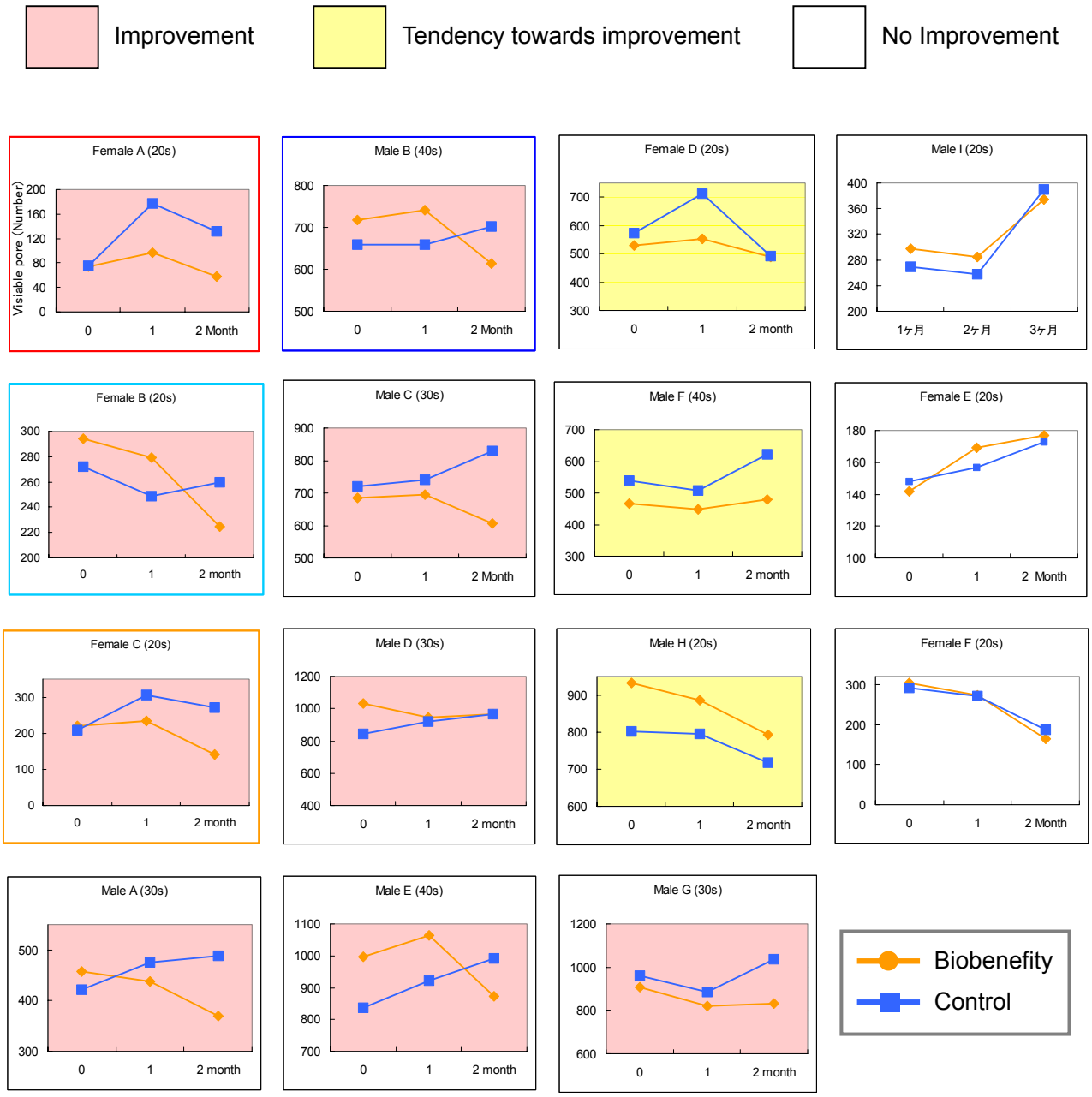


Fig. 20 Improvement of skin pore

(Some frame color is forward to page 18 and 19.)

Stability Test

Stability of Biobenefity was investigated.

1.Long Term Stability

Store Biobenefity in a cool dark place(4℃), room temperature, window side and at 50℃. Absorbance value and concentration of cynaropicrin at 410nm were determined.

Result and Discussion

Change of Absorbance value at 410 is shown in Fig. 21 and concentration of cynaropicrin is shown in Fig.22. Biobenefity is stable at any condition.

According to this result, Biobenefity does not have time dependent change and is significantly stable product.

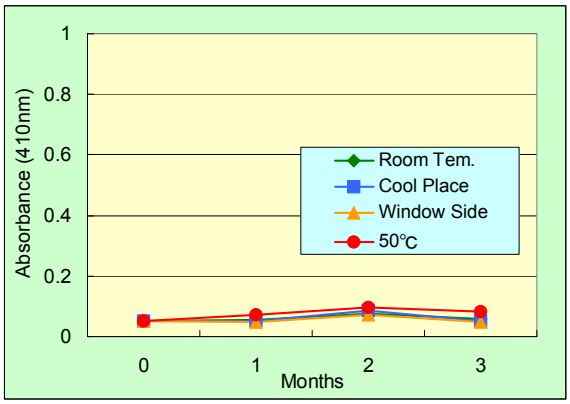


Fig.21 Long term stability 1

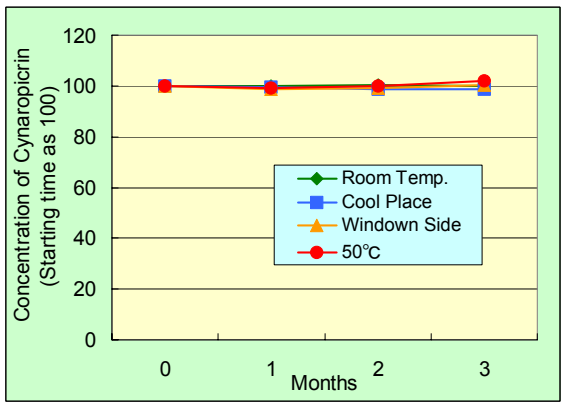


Fig.22 Long term stability 2

2. Thermal stability

Biobenefity was heated in a water bath at 90℃. After cooling down, absorbance value was measured at 410 nm.

Result and discussion

Thermal stability of Biobenefity is shown in Fig. 23. The increase of absorbance of Biobenefity and precipitate were not observed. According to the result, Biobenefity is significantly stable for short heating.

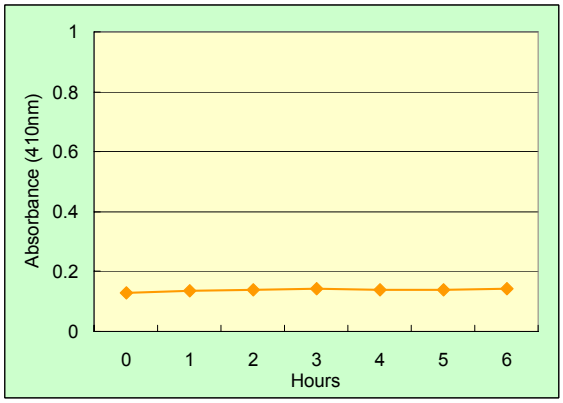


Fig.23 Thermal Stability

3.pH Stability

pH of Biobenefity is adjusted from 3 to 10 by HCl and NaOH. Absorbance value at 410nm were determined.

Result and Discussion

Absorbance value of Biobenefity is shown in Fig.24 and color tone is shown in Fig.25. pH 3 to 8, it was almost stable on color, but over pH8 the color was darkened. Color tone was also changed a little by little. Precipitation was not observed at any pH range.

According to the result, Biobenefity is necessary to take care at alkali range.

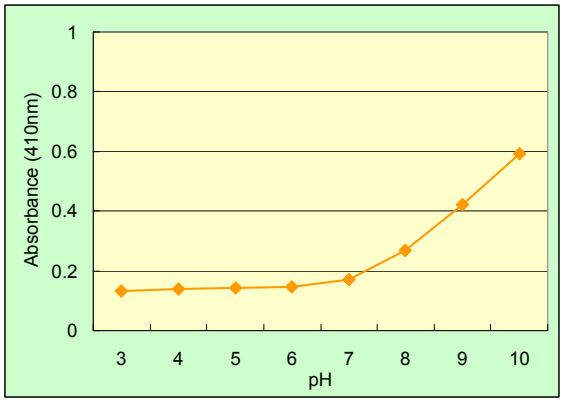


Fig.24 pH Stability

Compatibility Test

Compatibility of Biobenefity with various ingredients was evaluated.

Test Method

Biobenefity was diluted to 5 %, and the test samples were adjusted by purified water as shown on the each table. After 24 hours later, mixed solution was evaluated.

Result and Discussion

Results are shown on table 1 to 3.

1.Compatibility of 5% of Biobenefity with Surfactant

	%	Ingredients	Result
Cation	2.8	Stearyl Trimethyl Ammonium Chloride	○
	3.0	Cetyltrimehylammonium Chloride	○
	2.7	Lauryltrimethylammonium Chloride	○
Anion	10.0	Triethanolamine Lauryl Sulfate	○
	25.0	Sodium Laureth Sulfate	○
	25.0	Triethanolamine Laureth Sulfate	○
	6.25	Laureth-6 Carboxylic Acid	○
	10.0	Sodium N-Cocoyl-N-methyl Taurate	○
	10.0	Potassium N-Cocoyl Glycinate	○
	7.5	Sodium Lauroyl Methylaminopropionate	○
	25.0	Sodium Tetradecenesulfonate	○
Nonion	10.0	Polyethylene Glycol (50) Oleyl Ether	○
	10.0	Coconut Tatty Acid Diethanolamide	○
	10.0	Sorbeth-60 Tetraoleate	○
	10.0	Polyoxyethylene Sorbitan Monooleate (20E.O.)	○
	10.0	Polyoxyethylene Hydrogenated Castor Oil (60E.O.)	○
Silicone	10.0	Polyoxyethylene・Methylpolysiloxane Copolymer	○
Ampholytic	3.5	Lauryl Dimethylaminoacetic Acid Betaine	○
	4.0	Sodium N-Cocoyl-N-Carboxymethyl-N-Hydroxyethyl Ethylenediamide	○
	2.9	Lauroyl Amide Propylhydroxysulfobetaine	○

○: Good, △: Slight Turbidity, ×: Precipitate

2.Compatibility of 5% of Biobenefity with other ingredients

	%	Ingredients	Result
Solvent	50	Glycerin	○
	50	1,3-Butylene Glycol	○
	50	Propylene Glycol	○
	50	Isopropyl Alcohol	○
	50	Ethanol	○
Synthetic polymer	0.1	Carboxyvinyl polymer	○
	1	Polyvinylpyrrolidone	○
	1	Polyvinyl Alcohol	○
	1	Polyethylene glycol (6000)	○
Natural polymer	1	Sodium alginate	○
	1	Carboxymethyl cellulose	○
	1	Cationic cellulose	○
	0.1	Bio Sodium Hyaluronate HA12	○
	1	Hydroxypropyl cellulose	○
Phospholipid	1	Lipidure-PMB	○
Vitamin-C derivative	2	Ascorbyl Glucoside	○
	2	Pacificos VAP	○

○: Good, △: Slight Turbidity, ×: Precipitate

3.Compatibility of 5% Biobenefity with other our products

%	Product name	INCI Name	Result
5	FM Extract LA-B	Lactobacillus / Milk Ferment Filtrate	○
5	Absorage	Plantago Major Seed Extract	○
5	OUGON Liquid B	Scutellaria Baicalensis Root Extract	○
5	Caffenoage	Coffea Arabica (Coffee) Seed Extract	○
5	CHITIN Liquid (N)	Carboxymethyl Chitin	○
5	HPCH Liquid	Hydroxypropyl Chitosan	○
5	CureBerry	Vaccinium Myrtillus Leaf Extract	
5	Clairju	Hydrolyzed Prunus Domestica	○
5	KOTHALAHIMBUTU Liquid B	Salacia Reticulata Wood Extract	○
5	SAKURA Extract B	Prunus Yedoensis Leaf Extract	○
5	MARINWORT IPC-14 SBW	Algae Extract	○
5	SILKGEN G Soluble	Hydrolyzed Silk	○
5	SILKGEN G Soluble-S	Hydrolyzed Silk	○
5	TREHALOSE 30	Trehalose	○
5	NEEM Leaf Liquid B	Melia Azadirachta Leaf Extract	○
0.1	Bio-PHA Na Powder	Polyglutamic Acid	○
5	Bio-PGA Solution HB	Polyglutamic Acid	○
5	Bio-PGA Solution LB	Polyglutamic Acid	○
5	Bio antiage B	Pueraria Lobata Root Extract Chlorella Vulgaris Extract Aloe Barbadensis Leaf Extract	○
5	PEACH Leaf Liquid B	Prunus Persica (Peach) Leaf Extract	○
5	Biocellact ALOE VERA B	Aloe Barbadensis Leaf Extract	○
5	Fermentage Chardonnary B	Lactobacillus/Grape Juice Ferment	○
5	Fermentage Pear B	Lactobacillus/Pyrus Communis (Pear) Fruit Juice Ferment	○
5	Pharconix CTP-F (BG)	Hydrolyzed Collagen	○
5	JIOU Liquid	Rehmannia Chinensis Root Extract	○
5	SOUHAKUHI Liquid (BG)	Morus Alba Root Extract	○
5	HIOUGI Liquid	Belamcanda Chinensis Root Extract	○
5	BOTANPI Liquid E	Paeonia Suffruticosa Root Extract	○
5	LEMONGRASS Liquid B	Cymbopogon Schoenanthus Extract	○
5	Phyto COLLAGE (N)	Natto Gum	○
5	Phyto HYALURON B	Hibiscus Esculentus Fruit Extract	○
5	FLAVOSTERONE SB	Glycine Soja (Soybean) Protein	○
5	PrinessCare	Geranium Robertianum Extract	○
5	YUZU Ceramide B	Citrus Junos Fruit Extract	○
5	LACTOSACCHARIDES B	Yogurt Filtrate	○
5	RYOKUCHA Liquid	Camellia Sinensis Leaf Extract	○
5	LUNAWHITE B	Oenothera Biennis (Evening Primrose) Seed Extract	○

○: Good, △: Slight Turbidity, ×: Precipitate

Specification

Subject	Specification
Appearance	Light brown to yellowish brown liquid, having characteristic odor.
Identification Terpenoid	 Positive
Purity Heavy metals Arsenic	 20 ppm max. 2 ppm max.
Residue on Evaporation	0.18 w/v% min.
INCI Name	Butylene Glycol Water Cynara Scolymus (Artichoke) Leaf Extract
CAS Number	84012-14-6
EINECS Number	281-659-3

Additional document

Mechanism of Cynaropicrin (Biobenefity)[18)]

The action mechanism of the inhibitory effect of cynaropicrin, an active component of Biobenefity, on NF- κ B actions was analyzed. Cynaropicrin showed the inhibitory effect on an increase in TNF-α dependent NF- κ B transcription. (*2) Since TNF- α dependent NF- κ B transcription is increased by an increase in phosphorylation of I- κ B (*1) (Fig. 26), the action site of cynaropicrin was presumed to be the downstream of this step. Thus using the cells transfected with p65 gene (*3) to express active NF- κ B, the action of cynaropicrin on the steps after activation of NF- κ B was examined. As the result, cynaropicrin inhibited the increase in NF-κB transcription even in the cells strongly expressing active NF- κ B. The inhibitory degree was almost the same as that in TNF- α dependent NF- κ B transcription (Fig. 27), suggesting that the inhibitory action of cynaropicrin on the increase in NF- κ B transcription occurs mainly at the steps after activation of NF- κ B.

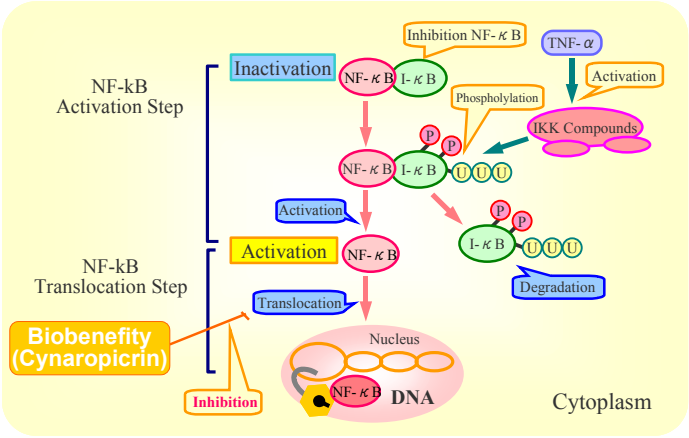


Fig.26 Active process of NF- κ B and Inhibitory process of Biobenefity

Using an immune staining method, the intracellular localization of NF- κ B at the increase in TNF- α dependent NF- κ B transcription was examined. The histological image revealed that the transfer of cytoplasmic NF- κ B to the nucleus by stimulation of TNF- α was blocked by cynaropicrin (Fig. 28). Thus it was cleared that the inhibitory action of cynaropicrin, namely Biobenefity on the increase in NF- κ B transcription was produced by blocking the transfer of active NF- κ B into the nucleus (Fig. 26).

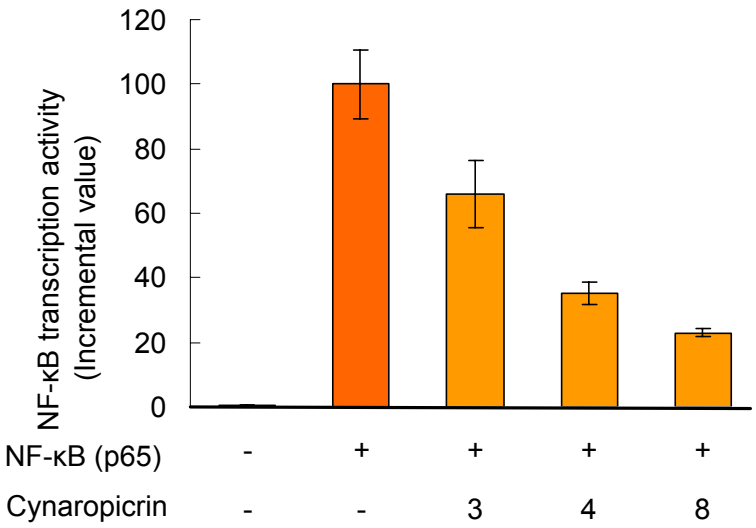


Fig.27 Inhibition of NF-κB hypertranscription of cynaropicrin by express cell of active NF-κB

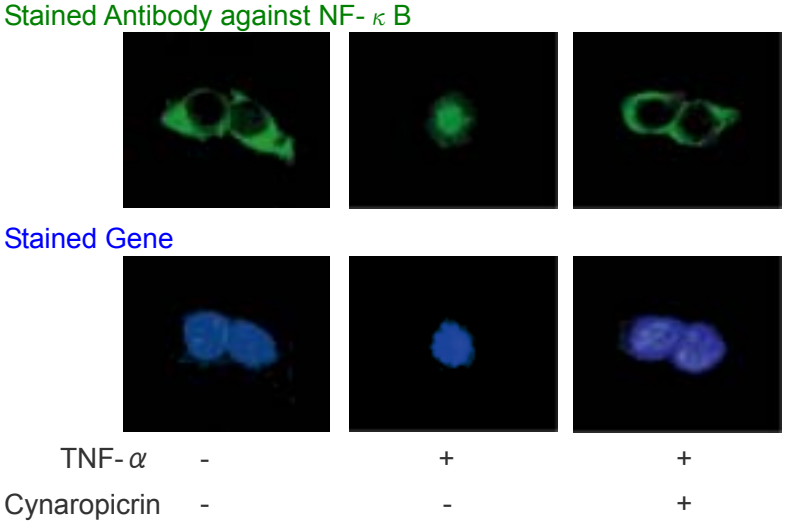


Fig.28 Transfer NF-κB into nuclear and inhibition by cynaropicrin

*1: It has been said that 1-κB blocks the transfer of NF-κB into the nucleus by binding with NF-κB.

*2: Degradation of I-κB is induced by signal phosphorylation by IKK complex.

*3: NF-κB is a dimer of p65 and a cells transfected with p65 gene expresses a large amount of active NF-κB.

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