

Original Article / 원저

Gagamyeonryunggobon-dan(*Jiājiǎnyánlínggùběn-dān*) induces hair regrowth effect from activating hair follicle

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모낭 활성화를 통한 가감연령고본단의 모발 발모 효과

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Abstract

Objective : The purpose of this study is to report the hair regrowth effect of *Gagamyeonryunggobon-dan* on ICR mice from measuring the change of diverse factors.

Methods : *Gagamyeonryunggobon-dan* was treated by oral administration with 2.5mg/kg/day amount for 3 weeks per mouse everyday. Hair regrowth was estimated by change of morphology, angiogenesis, hair follicle activation. The change of morphology was observed with external, internal change and sebaceous gland. Angiogenesis was estimated by image analysis, capillary distribution and angiogenic chemokine(MIP-2). Hair follicle activation was estimated by PCNA, IGF-2 and serotonin.

- Results** : 1. *Gagamyeonryunggobon-dan* treated group had more and thicker hairs than the group not treated. Especially well developed sebaceous glands were seen in dermis of treated group.
2. *Gagamyeonryunggobon-dan* treated group had more capillaries near hair follicles of subcutaneous layer and more 2019% MIP-2 positive activity than the group not treated.
3. *Gagamyeonryunggobon-dan* treated group increased positive activity up to 596% in PCNA, 187% in IGF-2 and 547% in serotonin more than the group not treated.

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Conclusion : These results shows that *Gagamyeonryungobon-dan* have the hair regrowth effect through verifying change of morphology, angiogenesis, chemokines. Consequently *Gagamyeonryungobon-dan* is expected to apply to take care of extensive hair loss symptoms.

Key words : *Gagamyeonryungobon-dan*, hair regrowth; hair follicle; angiogenesis

Introduction

Recently in Korea, the number of young alopecia patients in 20s and 30s are increasing faster than ever due to social and psychological reasons. It is known that more than 70% male persons over the age of 20s have worried about their hair loss¹⁾. It is assumed that 20% of them are actual alopecia²⁾. The alopecia doesn't harm a human vital activity. But in generally, people are tend to be aware of their hair as the first means to represent their appearance along with their face³⁾, so hair affects significantly to make people attractive to the others. Socially many people think that it is possible to be interpersonally discriminated against if they doesn't have a good appearance⁴⁾. Therefore the people who suffering from alopecia feel more depressed and stressful than who are not. Especially male alopecia patients have low self esteem⁵⁾ and suffer from the stress of alopecia much more than that of their work life³⁾. Even, in the past it was thought that the alopecia was the symptom only for the elderly, whereas in recent the age proportion of initial alopecia is largest at 20s and 30s⁶⁾. The number of acquired alopecia patients are

increasing as well though they have no genetical alopecia factor at all⁵⁾.

In oriental medicine, the hair is mediated by the kidneys. If the blood is abundant, the hair is growing well, whereas if the blood is deficient, the hair is lost. So the hair is related deeply with the kidney and blood in oriental medicine⁷⁾. The hair is lost when the qi and blood on the Gallbladder and Kidney meridian are deficient⁸⁾. The hair is the rest of the blood, then the hair is glossy when the blood is abundant, is crumble when the blood is deficient, turns yellow when the blood gets burnt and turns white when the blood gets harmed⁹⁾. Like this, in oriental medicine the hair loss is the problem of the blood and kidneys. Moreover, causes of hair loss in oriental medicine are including the weakness of qi and blood, the deficiency of essence and blood, the heat, the anger and the overeating of sweet food¹⁰⁻¹⁴⁾. These causes are seems similar to the causes of hair loss in modern society like work life, stress, living habits⁶⁾. However, in oriental medicine treatments of hair loss or alopecia are much less than those of muscular skeletal disease in aspect of clinical availability¹⁵⁾. Therefore, the studies of alopecia treatments using oriental medicine are not enough.

The needs for studying treatments of alopecia are growing because young 20s and 30s alopecia patients are increasing and they are so stressful of their alopecia. Finasteride is well known as one of

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typical modern medicines for male alopecia, But it comes out the possibility to induce gynecomastia^{16,17)} and prostate cancer¹⁸⁾. Pyrimidine is used for partial liniment on alopecia scalp in modern medicine, But it is known that partially using pyrimidine only on the scalp could derive hypertrichosis on the whole body¹⁹⁾. Therefore alopecia patients' needs for oriental medicine treatment are increasing because of worrying about side effects of modern medicines, We tried to develop the oriental medicine that induces the regrowth of hair makes the hair glossy and abundant through improving the weakness of qi and blood and the deficiency of essence and blood what are causes of alopecia in oriental medicine.

Yeonryunggobon-dan is one of the oriental medicines to care for all kinds of deficiency with adding the essence and blood⁹⁾. So *Yeonryunggobon-dan* is well known for its effect to add the essence, blood and qi of liver and kidneys. Park et al.²⁰⁾ reported that *Yeonryunggobon-dan* had effects to delay aging on the population doubling number and the population time in fibroblasts, heart endothelial cells and mesangial cells. But they didn't reported its effect on hairs and hair follicles. Jeong et al.⁷⁾ already reported that *Yeonryungiksugobon-dan* based on *Yeonryunggobon-dan* had hair regrowth effect with affecting diverse immunohistochemical factors. But they didn't digitize how the factors were changed objectively.

Therefore, we intended to investigate the hair regrowth effect of *Gagameonryunggobon-dan*. We shaved the hair of ICR mice and then administered *Gagameonryunggobon-dan* to them,

Then external changes were observed with the unassisted eyes how hairs grew. The capillary distribution near the hair follicle was compared to figure out the effect. Additionally we measured significant changes of angiogenic chemokine and hair follicle activation chemokines.

Materials & Methods

I. Materials

A. Animal

8 week-old male ICR mice were purchased from the Korean laboratory animal center. Mice were selected with 20g body weight after adjusting to aseptic environment for 2 weeks. The selected mice were classified into the control group, shaved group(SM) and treated group with *Gagameonryunggobon-dan*(GM). 10 mice were assigned to each groups.

B. Shaving

First, SM and GM back hairs were shaved by a razor (Joas, Korea). Then hair removal cream (Sensitive, Korea) on shaved region were used for secondary shaving. With these processes, the follicles of mice were induced to new anagen totally different from spontaneously induced anagen.

C. Preparation of *Gagameonryunggobon-dan*

Gagameonryunggobon-dan contains 22 herbal medicine which were purchased from *Seonyakdang* (Uiwang, Korea) (Table 1). 210g of *Gagameonryunggobon-dan* was prepared in grinded powder of herb. 21g of the grinded

powder was boiled with 500ml distilled water for 2 hours and the boiled water was filtered. The filtered water was depressurized and increased into 50ml with rotary evaporator. Then increased one was fed to GM group by oral administration with 2.5mg/kg/day amount for 3 weeks per mouse everyday.

II. Methods

A. Preparation of Tissues

3 weeks later after shaving, mice were anesthetized with the solution of sodium pentobarbital. Then the dorsal skins were cut and fixed with 10% NBF for 24 hours at a room

temperature. The fixed tissues were embedded by paraffin and made to tissue sections of 5 μm thickness.

B. Observation of Sebaceous Gland

Masson trichrome staining was done to observe the change of sebaceous gland. Mordanting treatment in 50-60°C Bouin solution was held for an hour and picric acid was removed within 70% ethanol. Cell nucleus was stained in Weigert iron hematoxylin for 10 minutes. Sebaceous gland and collagen fibers were stained in Biebrich salet-acid fuchsin and phosphomolybdic-phosphotungstic acid for 15 minutes each and then in aniline blue for 5 minutes. Then, we observed the sebaceous gland(red) and collagen fibers(blue) with the optical microscope(BX50, Olympus, Japan)

Table 1. The Amount and Composition of *Gagameonryungobon-dan*

Scientific Name	Dose (g)
Achyranthis Bidebtatae Radix	140
Alismatis Rhizoma	140
Polygoni Multiflori Radix	125
Asparagi Tuber	125
Sophorae Radix	100
Biotae Semen	90
Lycii Fructus	90
Rubi Fructus	90
Zizyphi Spinosae Semen	85
Ginseng Radix	80
Plantaginis Semen	75
Fsicae Semen	75
Polygalae Radix	70
Mori Fructus	65
Cuscutae Semen	65
Drynariae Rhizoma	60
Anemarrhenae Rhizoma	55
Eucommiae Cortex	50
Ecliptae Herba	45
Poria	45
Chrysanthemi Flos	45
Epimedii Herba	20
Total	1735

C. Observation of Angiogenesis

1. Image Analysis of Angiogenesis

Dorsal skins were cut and drawn aside. Then we found capillaries and took a picture of them magnified by a factor of 4. Capillaries looked clearer with Sharpen low - filter on image function of Image pro Plus (Media Cybernetic, USA). After that, capillaries were inverted and intensified to intensity 180-200 with inverting function on binary morphology.

2. Change of Capillary Distribution

Tissues were stained by Phloxine-tartrazine to observe the change of capillaries distribution near hair follicles on the subcutaneous layer. Nucleus was stained in Mayer's hematoxylin for 5 minutes and made

to react with phloxine solution for 30 minutes. Then, we observed that after distinguishing in tartrazine solution,

3. Change of Angiogenic Chemokine

Macrophage inflammatory protein (MIP)-2 what relates to angiogenesis, is similar chemokine to IL-8. We stained MIP-2 immunohistochemically to observe its distribution. Proteolysis of tissue sections was done in proteinase K (20 $\mu\text{g/ml}$) for 5 minutes. After proteolysis, tissue sections were reacted in blocking serum (10% normal goat serum) for 2 hours, then were reacted with mouse anti-Mip-2 (1:100, Santa Cruz Biotech) what is a primary antibody in 4°C humidified chamber for 72 hours. Furthermore, those were linked with biotinylated goat anti-mouse IgG_{2a} (1:100, DAKO, USA) what is a secondary antibody for 24 hours at room temperature, then were reacted with avidin biotin complex kit (Vector Lab, USA) for 1 hour at room temperature. We made them color in 0,05M tris-HCl (pH 7,4) buffering solution that contained 0,05% 3,3'-diaminobenzidine and 0,01% HCl, and counterstain with hematoxylin. Through the image analysis, we calculated the reaction of MIP-2 in subcutaneous layer.

D. Chemokine of Indicating Hair Follicle Activation

1. Mitosis

Proliferating cell nuclear antigen (PCNA) what is made on the early phase of mitosis, was observed to investigate change of its

distribution in hair follicle. It was stained immunohistochemically with rabbit anti-PCNA antibody and compared to each groups by image analysis.

2. Growth factor

Insulin-like growth factor (IGF) - 2 what is one of the growth hormones, was observed to investigate change of its distribution near hair follicle. It was stained immunohistochemically with goat anti-IGF 2 antibody and compared to each groups by image analysis.

3. Neurotransmitter

Serotonin what is one of the neurotransmitters, was observed to investigate change of its distribution near hair follicle. It was stained immunohistochemically with rabbit anti 5-HT antibody and compared to each groups by image analysis.

E. Image and Statistical Analysis

Image pro Plus (Media Cybernetic, USA) was used to analyze images and digitize immunohistochemical results. The results was analyzed with SPSS 22.0. After normality test, the results did not follow normal distribution. So, the results was tested with *Mann-Whitney* test. The statistical significance was attained when a p-value was less than 0,05.

Results

I. Change of Morphology

A. External Morphology

GM group was seen more hairs on their dorsal

skin than SM group. In addition to, GM group had thicker hairs than SM group(Fig. 1A, B).

B. Internal Morphology

GM group had wider subcutaneous layer and more hair follicles than SM group. In addition, GM group had lower density of collagen fiber in dermis and subcutaneous layer than SM group(Fig. 1C, D).

C. Sebaceous Gland

GM group had more sebaceous glands in dermis than SM group. Moreover, the evidence of complex lipid mixture was found more definitely in GM group than SM group(Fig. 1E, F).

II. Angiogenesis

A. External Observation

GM group had more capillaries than SM group. The thicker capillaries were found more in GM group than SM group(Fig. 2A, B).

B. Capillary Distribution

After Phloxine-tartrazine staining, GM group had more capillaries near hair follicles than SM group. In addition, strong positive reaction was found in internal root sheath(Fig. 2C, D).

C. Angiogenic Chemokine

After MIP-2 immunohistochemical test, positive

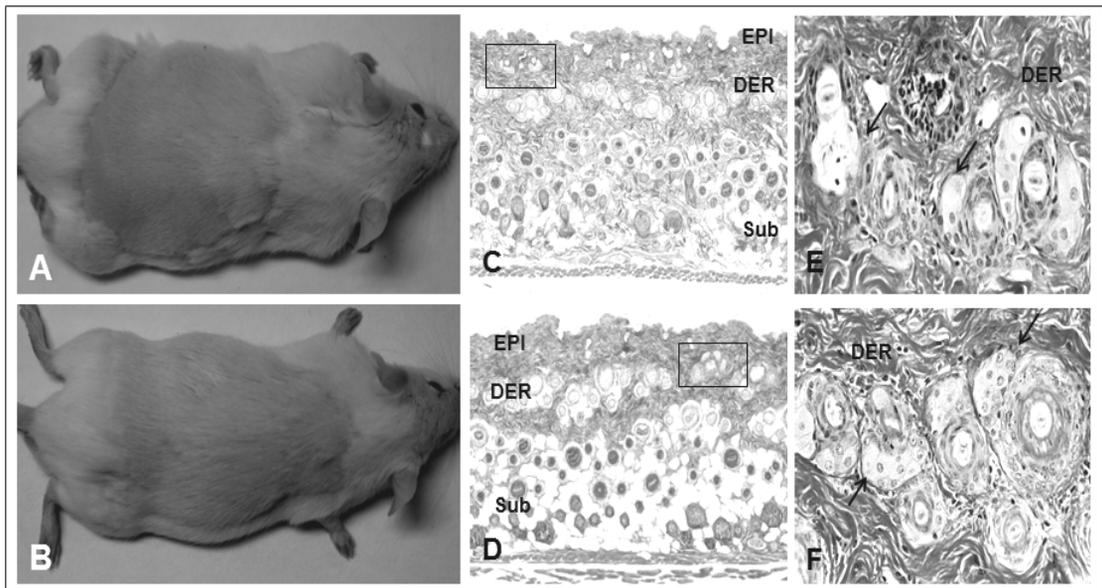


Fig. 1. General morphologic changes. A. Shaved mouse (SM). B. *Gagameonryunggobon-dan* treated mouse (GM). C. Skin structure in SM (x40). D. Skin structure in GM (x40). The enlargement of subcutaneous layer was appeared. E. Magnification of sebaceous gland (arrow) in C-square (x400). F. Magnification of sebaceous gland in D-square (x400). The inclusion of gland were increased than E. (x400) C, D, E, and F stained by Masson trichrome method. Abbreviation, EPI, epithelium; DER, dermis; SUB, subcutaneous layer.

MIP-2 was found in internal root sheath of hair follicle on subcutaneous layer. Positive MIP-2 reaction was increased by 2019% in GM group compared to SM group(Fig. 2E, F, Table 2).

III. Chemokine of Indicating Hair Follicle Activation

A. Mitosis

After PCNA immunohistochemical test, positive PCNA was found in internal root sheath of hair follicle on subcutaneous layer. Positive PCNA reaction was increased by 596% in GM group compared to SM group(Fig. 3A, B, Table 2).

B. Growth Factor

After IGF-2 immunohistochemical test, positive

IGF-2 was found in internal root sheath of hair follicle on subcutaneous layer. Positive MIP-2 reaction was increased by 187% in GM group compared to SM group(Fig. 3C, D, Table 2).

C. Neurotransmitter

After serotonin immunohistochemical test, positive serotonin was found in internal root sheath of hair follicle on subcutaneous layer. Positive serotonin reaction was increased by 547% in GM group compared to SM group(Fig. 3E, F, Table 2).

Discussion

Hair and hair follicle are kinds of skin

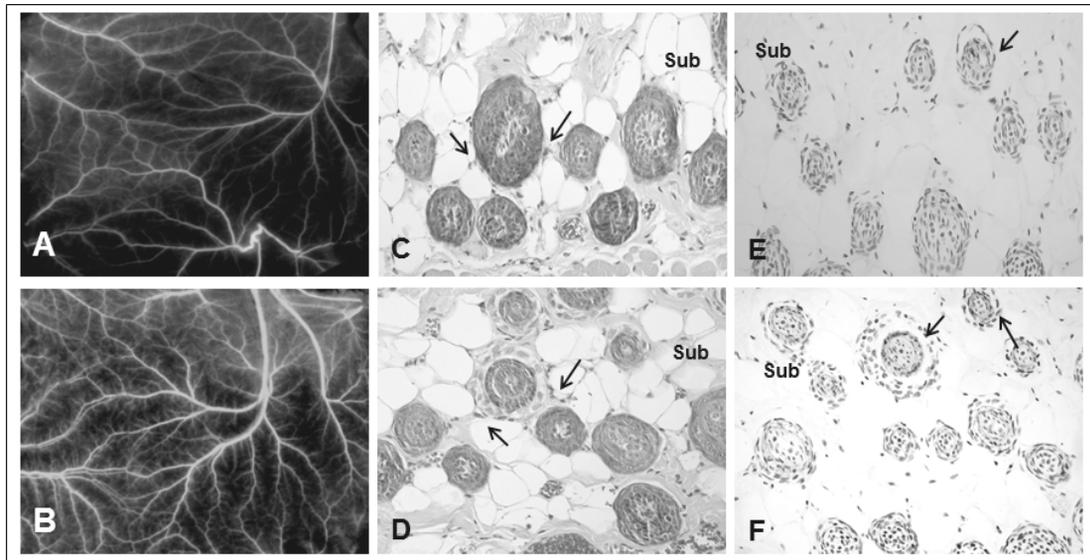


Fig. 2. Angiogenic changes. A. External vessel distribution in SM (x4). B. External vessel distribution in GM (x4). C. Hair follicle around capillary in subcutaneous layer of SM (x400). D. Increased hair follicle around capillary in subcutaneous layer of GM (x400). E. MIP-2 (arrow) immunohistochemistry in SM (x400). F. Increase of MIP-2 positive reaction in GM (x400). C & D stained by Phloxine-tartrazine method.

appendages, Hair follicle is the skin appendage only for mammals and has mainly cosmetic functions rather than vital activity²¹⁾. On average, people have 100,000 ~ 120,000 hairs on their scalp. It's on the normal rage that 50~100 hairs are lost everyday. Hair loss is depend on seasons, ages and physical condition²²⁾. Alopecia is hair

loss occurs on the region where normally hairs keep growing up²³⁾. Generally alopecia occurs on the scalp but sometimes could occur on the trunk,

Hair follicle is a fundamental unit for making hairs, Hair bulb is on the bottom of matured hair follicle. In hair bulb, hairs are made and grown

Table 2. The Image Analysis of Hair Development in Shaved Skin

Objective	SM [*]	Group GM [†]	P-value
MIP-2 [‡]	355±18	7779±277	<0,001
PCNA [§]	1253±171	8715±310	<0,001
IGF	2618±224	7525±285	<0,001
Serotonin	2376±178	15376±1634	<0,001

* SM : Shaved Mice

† GM : *Gagameonryunggobon-dan* Treated Mice after Shaving

‡ MIP-2 : Macrophage Inflammatory Protein

§ PCNA : Proliferating Cell Nuclear Antigen

|| IGF-2 : Insulin-like Growth Factors-2

P-value was calculated by *Mann-Whitney* test, Image Analysis for 10,000,000 Particles, Range of Intensity : 800-100

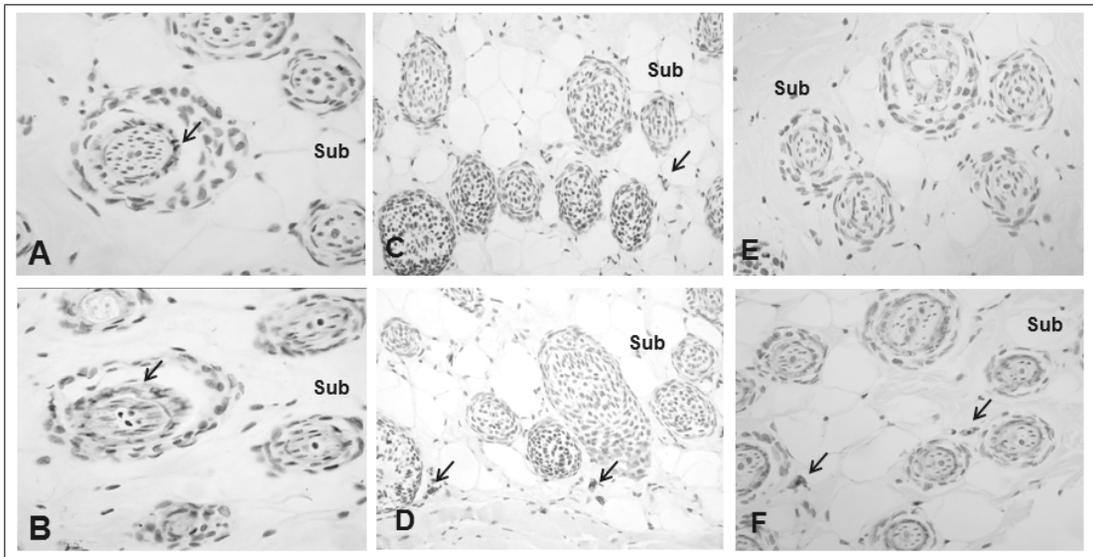


Fig. 3. Changes of Hair follicle activation, A. PCNA (arrow) immunohistochemistry in SM (x400). B. Increase of PCNA positive reaction in GM (x400). C. IGF-2 (arrow) immunohistochemistry in SM (x400). D. Increase of IGF-2 positive reaction in GM (x400). E. Serotonin (arrow) immunohistochemistry in SM (x400). F. Increase of Serotonin positive reaction in GM (x400).

up and there are a dermal papilla that has autonomic nerves and capillaries, and a hair matrix that occurs mitosis²⁴⁾.

Hair ontogeny has started since hair follicle was made for the first time. Hair formation occurs with the interaction between a dermal papilla and a hair matrix. If hair matrix stops mitosis or cell proliferation after dermal papilla shrinks, hair bulb becomes smaller and hair is isolated from hair bulb. Isolated hair is lost^{25,26)}. Mitosis in hair matrix occurs 10 times more than in basal cell of epidermis. If hair matrix gets harmed from cytostatic factor like anticancer drugs, it could lead to alopecia²⁴⁾. Therefore, it is important to activate mitosis and cell proliferation in hair matrix for hair growth. For activating mitosis and cell proliferation, dermal papilla is well supplied cytotrophy from capillaries around dermal papilla. If the blood supply around dermal papilla is not sufficient, the vitality of dermal papilla declines and the function of hair follicle is getting to lose²⁶⁾. Defunctionalization of hair follicle leads to alopecia.

The growth phase of hair is consist of anagen, catagen and telogen. Anagen is the phase that hair is growing up while mitosis occurs actively in hair matrix. During anagen, enough oxygen supply and cytotorophy are very important to activate hair follicle where mitosis takes place in rapidly. At the same time, they are determined according to whether angiogenesis is induced well near hair follicle. Hair growth and thickness are depend on how hair follicle are activated^{27,28)}. Especially during telogen, hair follicle secretes VEGF to activate angiogenesis that makes new anagen come up²⁹⁻³³⁾. If anagen is shortened and

telogen hairs are increasing, alopecia arises while new growing hairs are decreasing and lost hairs are increasing^{34,35)}. Shorten anagen and increased telogen hair could takes place when hair follicle has difficulty to exchange telogen to new anagen. Consequently inducing angiogenesis factors is a important progress for the regrowth of new hair on the way to make hair follicle exchange telogen to new anagen.

Finally, active mitosis in hair matrix, enough blood supply near hair follicle for active mitosis, and inducing angiogenesis factors to make hair follicle enter new anagen, these could be main factors to activate hair follicle leading to hair regrowth.

Yeonryunggobon-dan was reported that had effects to delay aging on fibroblasts, heart endothelial cells and mesangial cells by Parl et al.²⁰⁾. In addition to, it was known that *Yeonryunggobon-dan* affected elder mice significantly to increase fertility³⁶⁾. Jeong et al.⁷⁾ was reported that *Yeonryungiksugobon-dan* based on *Yeonryunggobon-dan* had a significant effects of hair regrowth and increasing of EGF and VEGF to prescribed mice in the immuno-histochemical study. Therefore, we estimated *Yeonryunggobon-dan* had a hair regrowth effect through adding the essence, blood and qi of liver and kidneys.

Gagamyeonryunggobon-dan is base on *Yeonryunggobon-dan* adding *Achyranthis Bidebatae Radix*, *Polygoni Multiflori Radix*, *Sophorae Radix*, *Zizyphi Spinosae Semen*, *Persicae Semen*, *Mori Fructus*, *Drynariae Rhizoma*, *Anemarrhenae Rhizoma*, *Ecliptae Herba*, *Chrysanthemi Flos* and *Epimedii Herba*, *Polygoni*

Multiflori Radix was already known that had the effect to take care of alopecia³⁷⁾. Achyranthis Bidebtatae Radix³⁸⁻⁴⁰⁾ and Mori Fructus⁴¹⁾ were reported to improve blood circulation, flow and reproduction, Drynariae Rhizoma⁴²⁾ and Sophorae Radix⁴³⁾ were reported that had a significant effect of hair growing and regrowth. As a result, *Gagameonryunggobon-dan* was made for improving blood supply near hair follicle and activating hair regrowth.

In this study, 10-week-old male ICR mice were tested. Mice were shaved on the back by a razor and then were applied hair removal cream to induce hair follicles to new anagen different from spontaneously induced anagen⁴⁴⁾. It was difficult to figure out hair regrowth index⁴⁵⁾ because ICR mice were albino. Therefore, hair regrowth effect was measured from activating factors what stimulated hair follicles.

It was observed visually that GM group had more hairs than SM group. In addition to, it was found that GM group had thicker and glossier hairs than SM group. In internal morphology, wider subcutaneous layer and more hair follicles were observed in GM group than SM group on that layer. On dermis, more sebaceous glands and evidence of complex lipid mixture were found in GM group than SM group.

There is a periodicity on the way of forming a hair. The bottom of hair follicle reaches subcutaneous layer during anagen whereas it reaches dermis during catagen and telogen²⁵⁾. It is suggested that more GM hair follicles reached subcutaneous layer than SM is not only to increase the number of GM hair follicles more than SM but also to increase anagen hair follicles

in GM group more than SM. Therefore, it shows that *Gagameonryunggobon-dan* could improve the hair regrowth effect with increasing the number of anagen hair follicles.

Sebaceous gland duct is connected to upper side of hair follicle. Cells of sebaceous gland proliferates and divides before secreting an oily matter called as sebum. Sebum is a kind of lipid complex mixtures consist of triglyceride, wax, squalene, cholesterol and cholesterol ester²⁶⁾. The lack of sebum makes hair dry because lipid membrane is not formed well to keep hair moisture from evaporating. It is suggested that the evidence of complex lipid mixture was found more definitely in GM group than SM is to secrete more sebum in GM group than SM. The activated sebaceous gland could make dry scalp improve. However, excessive secretion of sebum could cause seborrheic dermatitis. Therefore, additional researches are needed to figure out other effects of *Gagameonryunggobon-dan* that makes sebaceous gland activated.

The image analysis of capillary distribution were found that GM group had more and thicker capillaries than SM group. To make sure capillary distribution affect nearby hair follicles, Phloxine-tartrazine staining was conducted. As the result of staining, more capillaries near GM hair follicle was observed than near SM. Especially, strong positive reaction was found near inner root sheath of GM. It shows that *Gagameonryunggobon-dan* induces to activate forming capillaries near hair follicle and induced capillaries actually affects nearby hair follicles and inner root sheath. Inner root sheath is originated from hair matrix what develops to hair

substance²⁵⁾, so strong positive reaction of GM inner root sheath shows that *Gagamyeonryunggobon-dan* affect hair matrix activation leading to hair regrowth. In addition to, angiogenic factor would affect GM hair follicles with *Gagamyeonryunggobon-dan* to stimulate forming more capillaries.

MIP-2(Macrophage Inflammatory Protein-2) called as CXCL2 is one of CXC chemokine family. MIP-2 is well known for lipopolysaccharide what mainly secreted from injury and inflammation. It is also known its immunological interaction induced from TNF- α ⁴⁶⁾. Furthermore, MIP-2 is a marker that shows angiogenic activity along with VEGF and CCL2⁴⁷⁾. Therefore angiogenic activity can be measured from how much MIP-2 is activated.

As the result, MIP-2 of GM group had more 2019% positive reaction than that of SM. It shows that *Gagamyeonryunggobon-dan* could improve hair regrowth effects from promoting angiogenesis near hair follicle that makes hair follicle activated.

PCNA is an intercellular polymerase delta accessory protein of 36KD that unfound on G0 phase of cell cycle. It is increased on G1 phase, synthesized most on S phase and decreased on G2 phase. PCNA is an essential substance for measuring DNA synthesis and cell proliferation^{48,49)}. So, PCNA is used for one of molecular biological indexes that indicate how cell proliferates.

In inner root sheath of hair follicle, GM group had more 569% active reaction of PCNA than that of SM group. Inner root sheath is originated from hair matrix what develops to hair substance²⁵⁾, so more positive PCNA reaction of

GM indicates that more cell division and proliferation is occurred in GM hair matrix than in SM. Therefore, it shows that *Gagamyeonryunggobon-dan* could improve hair regrowth effects from promoting cell division of hair matrix and activation of hair follicle.

VEGF in dermal papilla of hair follicle is secreted during telogen while making hair follicle get into new anagen for activating angiogenesis²⁹⁻³³⁾.

IGF-2 (Insulin like Growth factor-2) is known as control factor for proliferation and division of diverse cells⁵⁰⁾. In addition, IGF-2 was already known as the factor that activates VEGF intensively⁵⁰⁻⁵²⁾. Therefore expression of IGF-2 in hair follicle could show how cell proliferates in hair follicle, how angiogenesis is activated by VEGF and whether hair follicles get into new anagen from VEGF activation.

IGF-2 near GM follicles are significantly measured more 187% activity than near SM. It show that *Gagamyeonryunggobon-dan* could improve hair regrowth effect from inducing cell proliferation near hair follicle and inducing VEGF to activate angiogenesis and hair follicles.

Serotonin is found not only in central nerve system but also in diverse cells like platelet and mast cell. Serotonin even has diverse effects as hormone, neurotransmitter, mitogen and etc. to almost whole cells^{53,54)}.

One of diverse serotonin effects is working directly on vascular endothelial cells and smooth muscles that leads blood vessels to contract or relax depends on kinds of serotonin receptors. Contracting blood vessels by serotonin is found in aorta, vena cave and venule. Relaxing blood vessels by serotonin is found in arteriole. So

serotonin near hair follicles has the effect of relaxation in arteriole, then relaxed arteriole increases blood supply in capillary⁵⁵⁾. Increased blood supply in capillary near hair follicle could affect generating thicker hair and extending the anagen phase similarly to topical pyrimidine derivatives application⁵⁶⁾. Therefore increasing serotonin near hair follicles could lead to more blood supply of capillaries and then increasing blood supply could activate hair follicle to have more effective hair regrowth, GM serotonin near hair follicle was significantly measured more 547% than SM. It shows that *Gagameonryunggobon-dan* could improve hair regrowth effect from increasing the density of serotonin near hair follicle that induces more blood supply in capillaries for activating hair follicles.

After all the results, GM group that took *Gagameonryunggobon-dan* had more effective hair regrowth than SM group that didn't take. It is suggested that *Gagameonryunggobon-dan* induces angiogenesis near hair follicles that stimulates hair follicles get into new anagen. In addition, the blood supply near hair follicles is increased as the result of angiogenesis. Increased blood supply to hair follicles activates hair follicle for increasing mitosis in hair matrix. It makes hairs much more and thicker. Through all these reactions, *Gagameonryunggobon-dan* is suggested to have hair regrowth effect and expected to apply to extensive hair loss symptoms for example telogen effluvium. It could be thought to apply not only when lost hairs are more than regrown ones but also when hairs are getting thinner. However, additional researches are needed to figure out the hair regrowth effect of

Gagameonryunggobon-dan on different pathologic hair loss such as androgenic alopecia and alopecia areata.

Unfortunately in this study, ICR mice were albino so that it was impossible to measure the hair regrowth index⁴⁵⁾ to calculate visually how much hair grown up. On the way of applying hair removal cream, SM group was also induced to new anagen in spite of not taking *Gagameonryunggobon-dan*. So all chemokines (MIP-2, IGF-2, PCNA and serotonin) were measured more in SM group than in control group that took nothing. It is thought that this fact will be investigated additionally from studying the effect of hair removal cream. In addition to, there was no study of *Gagameonryunggobon-dan* before this study so it is thought that diverse aspects of immunohistochemical studies are needed to figure out another chemokines or effects derived from *Gagameonryunggobon-dan*. It is also thought that constant try to develop new oriental medicines and remedies for alopecia is needed for many patients who suffer from alopecia.

Conclusions

This is the study for hair regrowth effects of *Gagameonryunggobon-dan* to activate hair follicle on shaved mice. In this study, *Gagameonryunggobon-dan*(1 g/kg/day) was taken to shaved mice for 3 weeks, then the state of hair growing, angiogenesis and hair follicle activation were investigated.

1. The group that *Gagamyeonryunggobon-dan* was taken had more and thicker hairs than the group not taken. Especially well developed sebaceous glands were seen in dermis of taking group.
2. The group that *Gagamyeonryunggobon-dan* was taken had more capillaries near hair follicles of subcutaneous layer and more 2019% MIP-2 positive activity than the group not taken.
3. The group that *Gagamyeonryunggobon-dan* was taken had more positive activity up to 596% in PCNA, 187% in IGF-2 and 547% in serotonin than the group not taken.

In conclusion, *Gagamyeonryunggobon-dan* should have the hair regrowth effect through inducing hair follicle activation. Consequently *Gagamyeonryunggobon-dan* is expected to apply to take care of extensive hair loss symptoms.

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