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An Optimal Combination of Inositol and Phytic Acid Effectively Promotes Hair Growth

Yuho Yamauchi, Toshio Nakamura*, Takuo Tsuno and Fumi Tsuno

TSUNO RICE FINE CHEMICALS Co., Ltd., Japan

***Corresponding author:** Toshio Nakamura, Senior Manager, Planning Development Dept. of Research and Development Div., Tsuno rice fine chemicals Co., Ltd, 94 Shinden, Katsuragi-cho, Ito-gun, Wakayama, Japan 649-7194

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ABSTRACT

Many people suffer from alopecia. The hair growth drug, minoxidil, stimulates vascular endothelial growth factor (VEGF) production as well as vascularization and hair growth. However, as minoxidil has side effects with long-term use in women, a safe alternative hair growth agent is needed. Therefore, we investigated whether inositol (IN) and phytic acid (PA), which are water-soluble components derived from rice bran, have a hair growth-promoting effect. Dermal papilla cells were cultured, and the gene expression level of VEGF was analysed using real-time PCR when IN and PA were added alone or in combination. Further, a clinical trial was conducted using a scalp lotion containing a mixture of IN and PA for women. The scalp lotion was applied twice a day for 18 weeks, and the hair density was measured. In cell-treatment experiments, the gene expression level of VEGF was increased by IN or PA and was further increased by their mixture in a 1:3 mass ratio. In a human study, hair density was significantly increased at 18 weeks after applying a scalp lotion containing IN and PA. Overall, IN and PA were effective in increasing the VEGF expression in dermal papilla cells and in increasing the number of hairs. This effect was synergistically enhanced when PA and IN were mixed in a 1:3 mass ratio. This combination of components is thus expected to lead to the development of safe and effective hair growth agents.

Keywords: Rice Bran; Inositol; Phytic Acid; Hair Growth; VEGF; IGF-1

Abbreviations: VEGF: Vascular Endothelial Growth Factor; IN: Inositol; PA: Phytic Acid; FGF: Fibroblast Growth Factor; IGF: Insulin Growth Factor; HFDPCs: Human Follicle Dermal Papilla Cells; GAPDH: Glyceraldehyde 3-Phosphate Dehydrogenase

Introduction

Many people suffer from alopecia because of aging, genetic predisposition, stress, and other causes. Epidemiological studies show that 85% of men and 40–50% of women are affected by alopecia during their lives [1]. Various attempts have thus been made to provide excellent hair growth agents. Minoxidil, a hair growth agent that stimulates vasodilation, is used to treat a wide variety of alopecia and telogen effluvium cases, including androgenetic alopecia in men and women. Previous studies have demonstrated that vascular endothelial growth factor (VEGF) stimulates hair growth by increasing hair follicular angiogenesis in mice [2]. Moreover, increased production of (1) VEGF, (2) fibroblast growth factor (FGF), and (3) insulin growth factor (IGF)-1 [3,4], all of which increase vascularization, is a part of minoxidil-mediated hair growth, suggesting that stimulation of vascularization and/or production of VEGF, FGF, and/or IGF-1 is a therapeutic strategy to treat various types of alopecia. However, because minoxidil has side effects with long-term use [5], a safe alternative hair growth agent is needed. Rice bran is a by-product of the process of milling brown rice to white rice, contains various functional ingredients. Its oil-soluble components include γ-oryzanol, ferulic acid, tocopherol, and tocotrienol, and its water-soluble components include inositol (IN) and phytic acid (PA) [6].

Much information regarding the safety of each of these components, including dietary and usage experience is available, and many reports on their physiological activity have been published. Among these, IN exists in cell membranes in the body mainly in the form of

inositol phospholipids. It exerts various physiological functions as a vitamin B-like substance. Phosphatidyl inositol is known to be degraded into inositol-3-phosphate and diacylglycerol upon activation of the receptors present in cell membranes by hormones and neurotransmitters, and to function as a second messenger in intracellular signal transduction pathways [7,8]. PA, also called inositol hexaphosphate is a phosphorylated compound with six phosphate groups attached to IN. We hypothesized that these components can exert hair growth-promoting effects. Therefore, we conducted a test to examine whether IN and PA, which are water-soluble components derived from rice bran, have hair growth-promoting effects.

Materials and Methods

The test subjects were rice bran-derived IN and PA commercialized by Tsuno food industrial Co., Ltd.

Cell Culture

Human follicle dermal papilla cells (HFDPCs) purchased from Promo Cell GmbH (Heidelberg, Germany) were grown in papilla cell growth medium (Follicle Dermal Papilla Cell Growth Medium (Ready-to-use); Prom Cell GmbH, Heidelberg, Germany), as described previously [9,10]. Cells were cultured at 1×10^5 cells/well in a 24-well plate or 1×10^4 cells/well in a 96-well plate in the medium, and incubated with IN or PA or their mixture (IP mix) for 24 h. Cell proliferation was measured using a CCK-8 kit (Dojindo Molecular Technologies, Inc., Kumamoto, Japan) with a water-soluble tetrazolium salt that produced orange formazan.

Quantitative Real-Time Polymerase Chain Reaction

Total RNA was isolated from cell lysates using the guanidine isothiocyanate–phenol–chloroform method [11] with ISOGEN (Nippon Gene Co., LTD., Tokyo, Japan), following the manufacturer's instructions. cDNA was then prepared using the PrimeScript RT Reagent Kit (TaKaRa, Kyoto, Japan). Real-time PCR was performed using the StepOne system (Thermo Fisher Scientific, Waltham, MA, USA) with specific primers. Real-time PCR was used detect the expression of glyceraldehyde 3-phosphate dehydrogenase (GAPDH), IGF-1, and VEGF using Power Up SYBR Green Master Mix (Takara Bio, Shiga, Japan). The expression levels of the target genes were calculated relative to the expression levels of the housekeeping gene GAPDH using the ΔΔCT method. Primer for GAPDH, VEGF and IGF-1 were designed based on reports by Adachi et al. (2015) [12] and Nakamura et al. (2018) [13] as follows; GAPDH, F5′-CTCCTGTTCGACAGTCAGCC-3′ and R5′-TCGCCCCACTTGATTTTGGA-3′, VEGF, F5′- CTACCTCCACCATGC-3′ and R5′- ATGATTCTGCCCTCCTCC-3′, and IGF-1, F5′- TTTCAAGCCACCCATTGACC-3′ and R5′- GCGGGTACAAGATAAATATCCAAAC-3′.

Human Clinical Study

Twenty-four healthy females suffering from thinning hair were recruited and randomly divided into two groups (12 per group, IP mix and placebo) at Nikoderm Research Inc. (Osaka, Japan). Finally, 23 participants were included and 1 participant (placebo group) was excluded as she declined participation owing to personal circumstances (Figure 1). A lotion containing IP mix or placebo was applied to the scalp for 18 weeks (Table 1). Participants did not use other hair growth reagents for at least 2 months before starting the study. The vertex scalp was photographed using an EOS Kiss X7 digital camera (Canon, Inc., Tokyo, Japan). The hair density and diameter were objectively assessed using phototrichogram [14] before, and 9 and 18 weeks after applying the test lotion. This study was planned according to the guidelines of the Declaration of Helsinki and Ethical Guidelines for Medical and Health Research Involving Human Subjects proposed by the Japan Ministry of Health, Labour, and Welfare. All participants provided written informed consent before commencement of the study, which was approved by the Ethics Committee at Nikoderm Research Inc.

Table 1: Placebo and IP mix formulation.

	Component
Placebo lotion	Water, butylene glycol, pentylene glycol, methylparaben, PPG-6—Decyltetradeceth-30, menthol, water, sodium hydroxide
IP mix lotion	Placebo lotion +0.81% phytic acid, 027% inositol

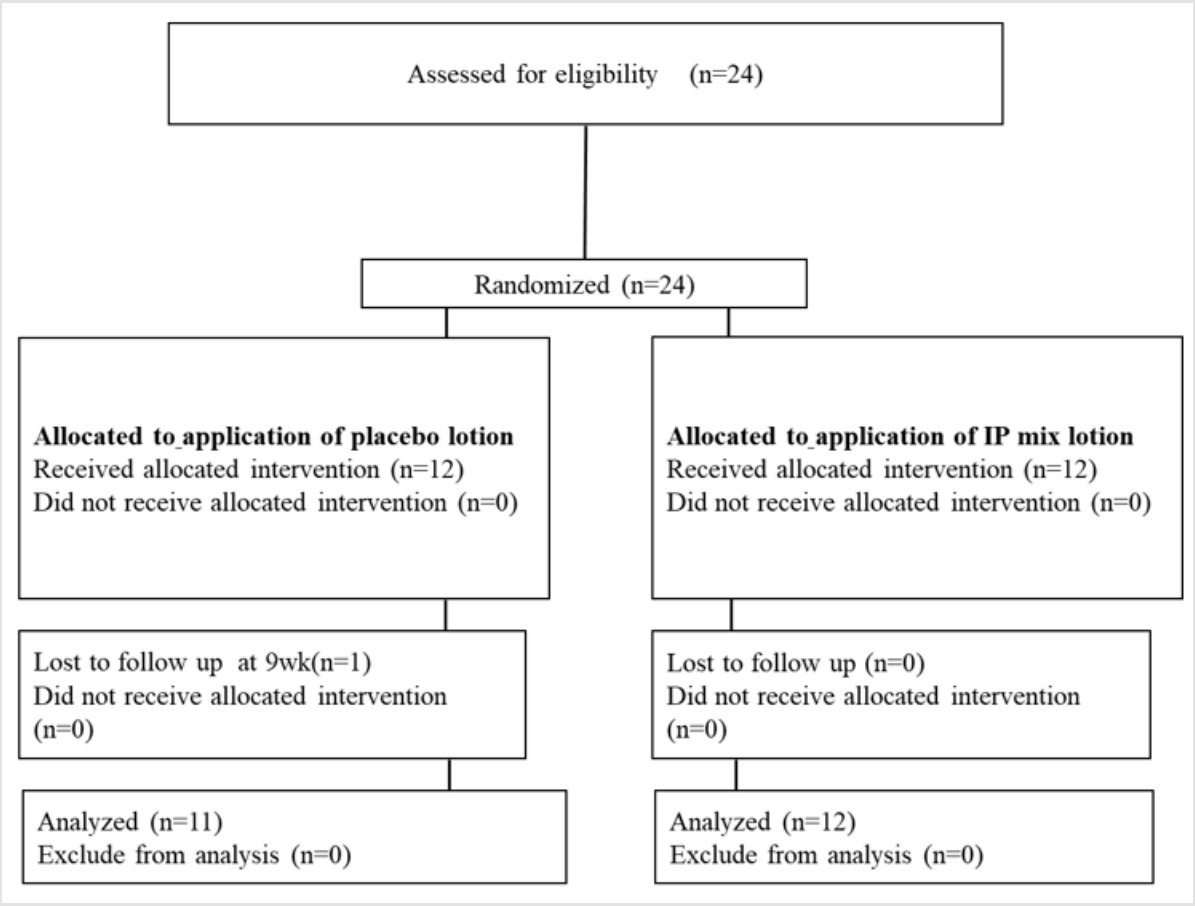

Figure 1: Study design: Two groups of female participants suffering from hair loss applied either the IP mix lotion or the placebo lotion.

Statistical Analysis

Results were statistically analysed using Dunnett's test or Tukey's test (in vitro cell culture study) and paired t-test and student unpaired t-test (human clinical study).

Results

HFDPC Cell Growth

HFDPC growth was significantly increased following incubation with 0.06–1% IN (Figure 2a) and 0.016–0.25% PA (Figure 2b) compared with that in the vehicle control.

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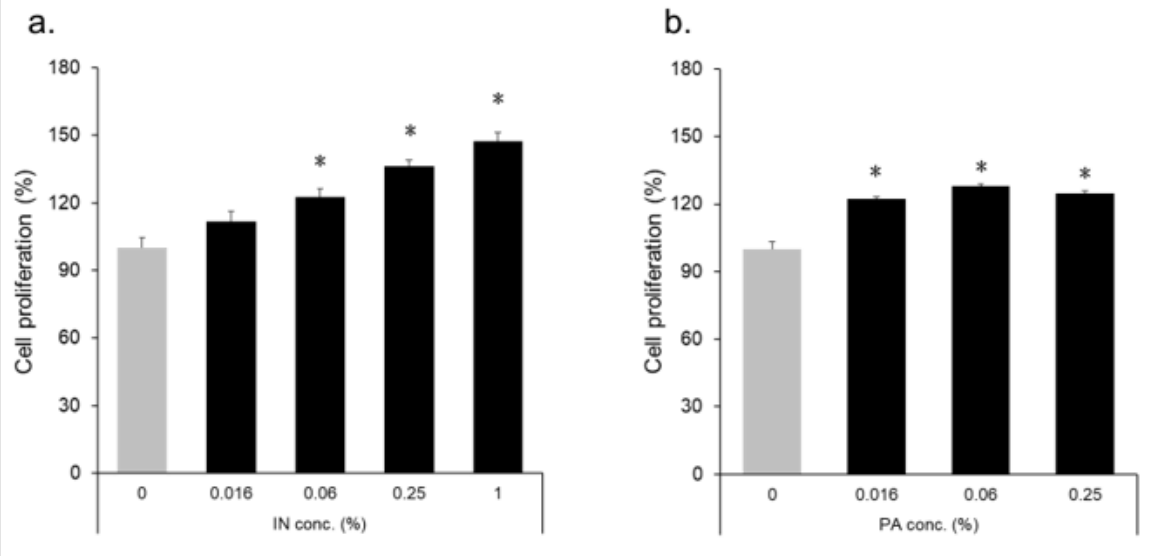

Figure 2: Growth of human follicle dermal papilla cells (HFDPC): HFDPCs were treated with inositol (a) Or phytic acid (b). Cell growth was measured using the CCK-8 Kit. Data are presented as the mean ± SE. (n = 5) Data were analysed using Dunnett's test. *; $p < 0.05$ (vs. 0%).

Gene Expression Levels of IGF-1 and VEGF in Cultured HF-DPCs

IGF-1 gene expression on RT-qPCR was significantly increased in cells treated with 0.5% PA, but not changed with IN, compared to that in the untreated cells (Figure 3). VEGF gene expression was significantly increased in cells treated with 0.5% IN and 0.5% PA compared with that in the untreated cells. Furthermore, the increase in VEGF gene expression was greater in cells treated with the IP mix, prepared with a 1:3 weight ratio of IN and PA, than in cells treated with IN and PA alone. (Figure 4a) Treatment with mixtures of IN and PA prepared at ratios of 1:2, 1:3, and 1:4 showed that the mixture with the 1:3 ratio was the most effective at increasing VEGF expression (Figure 4b).

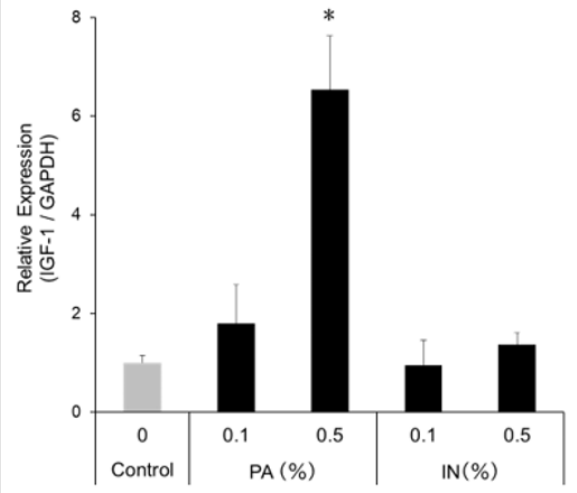

Figure 3: IGF-1 expression levels in human follicle dermal papilla cells (HFDPC) examined using real-time PCR: The IGF-1 expression level was determined relative to GAPDH expression, and the expression of untreated samples was denoted as 1. Data are presented as the mean ± SE. (n = 4) Data were analysed using Dunnett's test. *; $p < 0.05$ (vs. untreated samples).

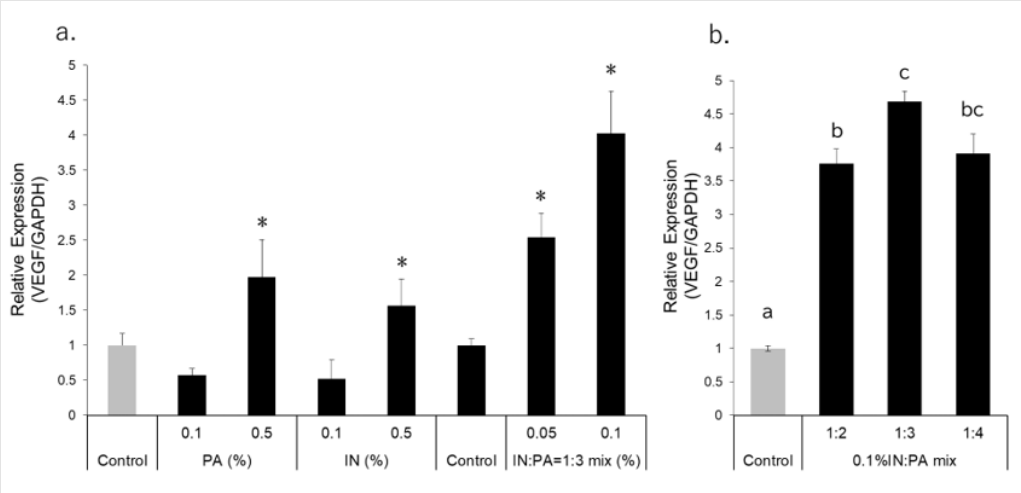

Figure 4: VEGF expression levels in human follicle dermal papilla cells (HFDPC) examined using real-time PCR.: The VEGF expression level was determined relative to GAPDH expression, and the expression of untreated samples was denoted as 1. Data are presented as the mean ± SE. (n = 4).

a. Data were analysed using Dunnett's test. *; $p < 0.05$ (vs. untreated samples)

b. Data were analysed using Tukey's test. There are significant differences between different alphabets. $p < 0.05$.

Hair Growth-Stimulating Effects of the IP Mix in Female Participants

We next assessed the effect of the IP mix with the 1:3 weight ratio of IN and PA, on hair growth in women. The mean age of the participants at the start of the study was 56.8 ± 4.8 years in the placebo group and 54.3 ± 7.7 years in the IP mix group. All participants applying the placebo and IP mix lotion completed the study without exhibiting any side effects such as hirsutism or facial hair growth during the study period. Phototrichogram analysis showed that hair density was significantly increased after applying the IP mix (Figure 5). However, the hair diameter of the IP mix group was slightly decreased (Figure 6a) and the rate of decrease in anagen hair was slower in the IP mix group than in the placebo group (Figure 6b). Photographing the vertex scalp also confirmed a grossly visible increase in hair density in the IP mix group (Figure 7). Participant A showed an increase in overall hair volume (Figure 7a). Participants B and C showed increased hair density in the areas indicated by arrows (Figures 7b & 7c); in Participant D showed a smaller width of the parting (Figure 7d) after 18 weeks of using the IP mix lotion.

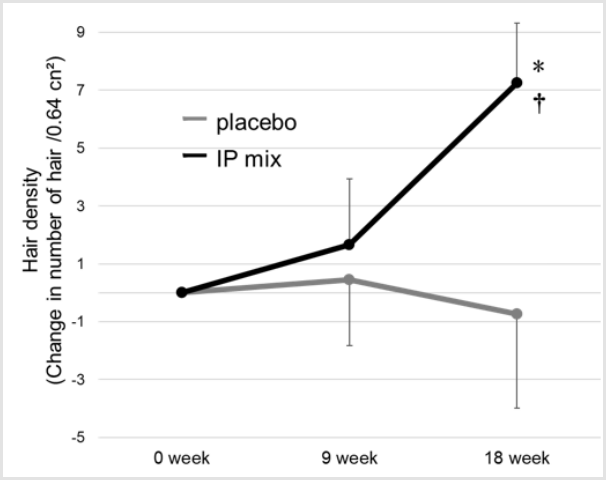

Figure 5: Changes in hair density from the 0th week in the clinical test: The grey line shows the placebo group (n = 11) and the black line (n =12) shows the IP mix group. Data are presented as the mean ± SE. Data were analysed using paired t-test and student unpaired t-test. *; $p < 0.05$ (*vs.* the 0th week), †; $p < 0.05$ (*vs.* the placebo-treated sides).

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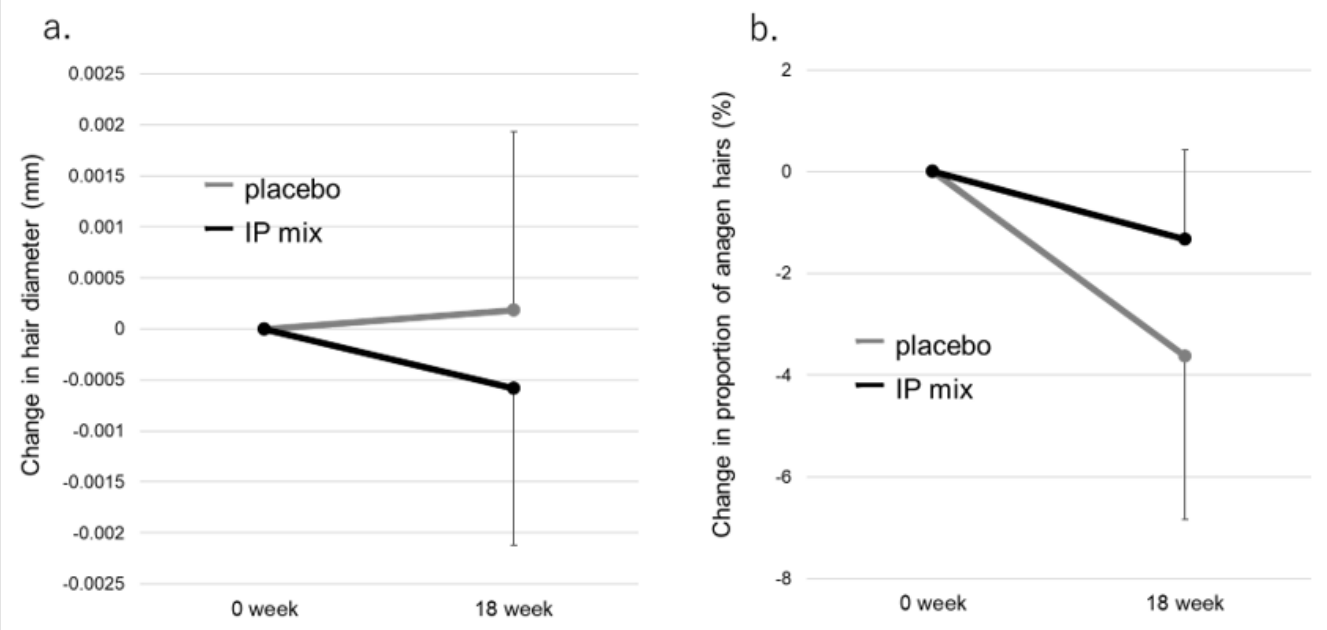

Figure 6: Changes in hair diameter and anagen hair from the 0th week in the clinical test. Figure 6a and 6b denote the hair diameter and anagen hair, respectively. The grey line shows the placebo group (n = 11) and the black line (n =12) shows the IP mix group. Data are presented as the mean ± SE.

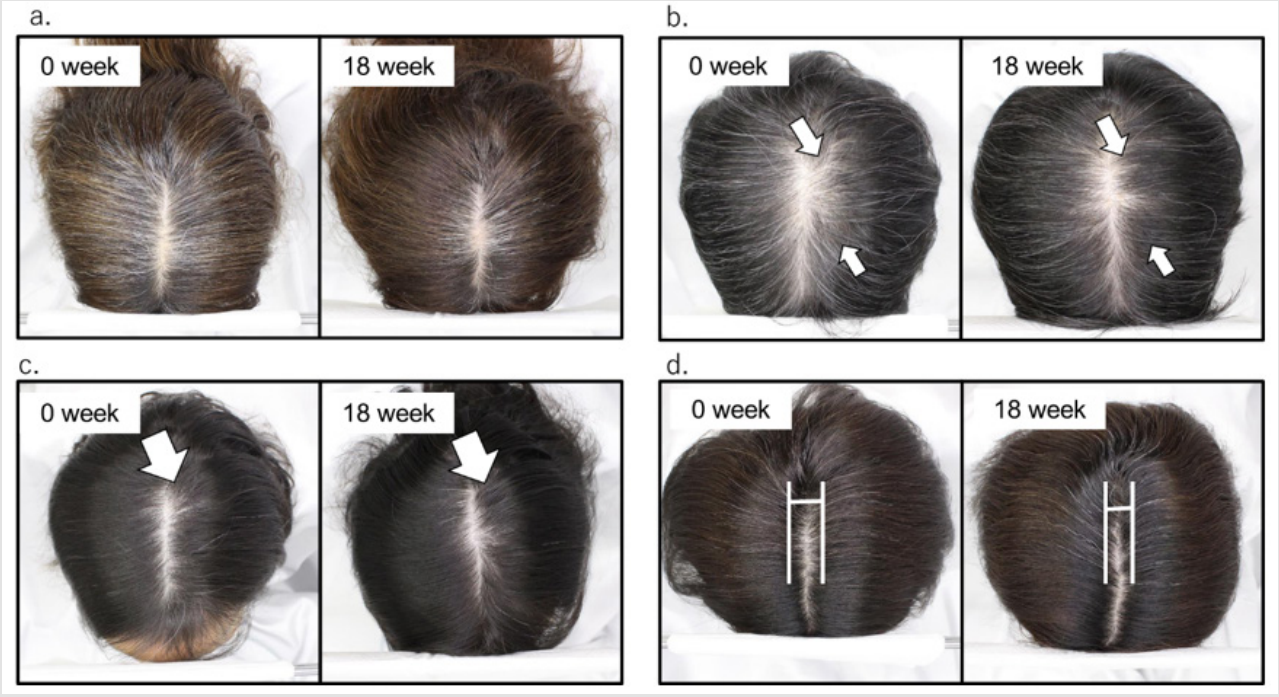

Figure 7: Photographs of the vertex participant scalp before and at 18 weeks after application of the IP mix lotion.

Discussion

IN and PA are contained in the seed coats of plants, such as in rice bran. Early growth of rice is known to be improved by using seeds with high PA content [15], and myo-inositol plays an important role in seed maturation and germination [16]. These components have thus been shown as factors involved in plant growth. In human cells, IN is a factor necessary for growth and survival [17], and inositol triphosphate, an intermediate between IN and PA, has many functions as an intracellular signal transmitter [18]. Therefore, we hypothesized that inositol and phytic acid can promote hair growth, and that these molecules may work synergistically. In this study, we demonstrated that IN and PA derived from rice bran stimulated VEGF production in HFDPCs. The effect of IN and PA was the strongest when mixed at a ratio of 1:3. Furthermore, the IP mix increased hair density in female participants. The decrease observed in hair diameter may be attributed to increased new hair growth. The anagen hair ratio was decreased (although not significantly) in the placebo group, whereas almost no change was found in the IP mix group, indicating that the decrease was suppressed by the IP mix.

Several studies have reported the synergistic effect of the combination of IN and PA in areas other than hair growth. When used in combination, IN and PA have been reported to effectively inhibit colorectal cancer progression more strongly compared to either agent alone [19]. Further, ingesting a mixture of IN and PA at a mass ratio of 220:800 (molar mass ratio 1:1) has been reported to improve abnormalities in carbohydrate and lipid metabolism [20]. In this study, the mixture mass ratio of IN and PA that produced the strongest effect was 1:3. The molar mass equivalent of this ratio is approximately 1:1, and total number of IN skeletons to phosphate groups is 1:3. The type 3 receptor for IP3, an inositol with three phosphate groups, is reported to be expressed in the hair follicles of the skin and to play an important role in regulating the hair cycle [21]. Additionally, inositol triphosphate receptor expression is increased by compounds that inhibit stress-induced damage in human hair follicles, suggesting a link with hair growth and hair loss [22]. These reports thus suggest that treatment with a mixture of IN and PA at a mass ratio of 1:3 (molar mass ratio of 1:1) can efficiently generate inositol triphosphate and potentially improve hair growth and prevent hair loss. In conclusion, this study demonstrated that the IN and PA mixture at 1:3 mass ratio increases VEGF production in HFDPCs. We further showed that the IN and PA mix improves hair growth in women. IN and PA are water-soluble ingredients derived from rice bran; therefore, they can be easily incorporated into dosage forms such as lotions. The combination of these ingredients is thus expected to contribute to the development of safe and effective hair growth agents.

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