의학박사 학위논문

Effects of polyunsaturated fatty acids on hair growth

고도불포화 지방산이 모발 성장에 미치는 영향

2015년 8월

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의학과 피부과학전공

Munkhbayar Semchin

A thesis of the Degree of Doctor of Philosophy

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Effects of polyunsaturated fatty acids on hair growth

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ABSTRACT

Effects of polyunsaturated fatty acids on hair growth

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Hair loss is a common problem but the treatments for the various forms of hair loss showed limited success yet. Polyunsaturated fatty acids (PUFAs) are long chain fatty acids with more than one C-C double bond in their backbones. From the ancient times, animal and plant oils, which have rich polyunsaturated fatty acids, have been used as a traditional remedy for treating hair loss in the several countries. Arachidonic acid (AA) and Eicosapentaenoic acid (EPA) are omega-6 and omega-3 fatty acid each and exert their effects through changes in membrane phospholipids or the production of signaling molecules such as eicosanoids.

The purpose of this study is evaluate effects of polyunsaturated fatty

acids on hair growth, and find out the possible mechanism of hair growth.

Therefore, we tested whether AA and EPA had effects on anagen induction or anagen prolongation using C57BL/6 mice. We also investigated the effect of AA on human dermal papilla cells (hDPCs) and in *ex vivo* organ culture model.

Anagen induction was tested with AA and EPA of 2% and AA plus EPA groups mixed with ratio of 1:1, 1:3, and 3:1, respectively. The regeneration of new hair coat was more prominently observed in 2% AA and AA plus EPA 3:1 treated group and moderately accelerated in 2% EPA and AA plus EPA 1:1, 1:3 group compared with control group. Furthermore, 2% AA and AA plus EPA 3:1 group markedly prolonged the anagen hair growth in an *in vivo* model of anagen prolongation induced by depilation.

AA significantly promoted elongation with increased proliferation of matrix keratinocytes *ex vivo* culture.

We found that AA enhanced the viability of hDPCs also promoted the expression of several factors that are known to promote hair growth, including fibroblast growth factor FGF-7, FGF-10 and hepatocyte growth factor.

Western blotting revealed that AA in hDPCs led to phosphorylation various transcription factors (ERK, CREB, and AKT) and increased the expression of Bcl2. These results suggest that PUFA plays role in hair growth. AA and EPA, both are polyunsaturated fatty acid as a

broad composition in plant and animal products, could be a potential candidate for the promotion of hair growth.

Key Words: Hair growth, Polyunsaturated fatty acid, Arachidonic acid, Eicosapentaenoic acid, Hair cycle, Human Dermal Papilla Cell

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INTRODUCTION

Polyunsaturated fatty acids (PUFAs) are long-chain fatty acids with more than one C–C double bond in their backbones. According to the position of the first double bond in the structure, PUFAs can be classified into two major categories, namely ω-3 and ω-6, which are present as essential cellular components and possess diverse biofunctions [1]. PUFAs are commonly believed to exert their effects through changes in membrane phospholipids or through the production of signaling molecules such as eicosanoids [2]. The (ω-6) (arachidonic acid: AA) and the (ω-3) (eicosapentaenoic acid: EPA) are polyunsaturated fatty acid that are present in an esterified form in all mammalian outer and intracellular membranes, where it is released from the membrane by enzyme phospholipases [3]. Free AA and EPA becomes available as a substrate for the intercellular biosynthesis of various different series of eicosanoids, such as prostaglandins (PGs), thromboxane (TX), leukotrienes (LTs), and hydroxyl eicosatetraenoic acids (HETEs), through the action of cyclooxygenases, lipoxygenases, and cytochrome P450, respectively

[3, 4]. As a result, AA and EPA, these eicosanoid metabolites are involved in the regulation of many cellular processes, such as cell survival, angiogenesis, chemotaxis, mitogenesis, apoptosis, and migration [5-9] .

The hair follicle (HF) is a mini organ that is unique to mammals, and a single HF can represent the full neuroectodermal-mesodermal interaction system. In a lifetime, HFs undergo cyclic transformations between rapid growth (anagen), regression (catagen), and a resting phase (telogen). In this context, the dermal papilla (DP) is composed of specialized group of fibroblasts that are located in the follicle's bulb, and is important in controlling hair growth and cycling [10-12].

Animal and plant oils, which are rich in polyunsaturated fatty acids, have been used in various countries as traditional remedies for skin irritations, rheumatic symptoms and hair loss. In addition, several case reports have described the topical application of safflower oil (which is rich in omega-6 polyunsaturated fatty acid, the linoleic acid-precursor of AA) resulted in the clinical improvement of scalp dermatitis, alopecia, and hair depigmentation; these conditions are symptoms of essential fatty acid deficiency syndrome [13, 14].

Moreover, several recent studies have demonstrated that hair growth can be promoted using latanoprost and isopropyl unoprostone, which are analogues of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$,the final metabolite of AA) [15]. However, the direct effects of AA and its eicosanoid metabolites on human dermal papilla cells (hDPCs) have yet to be reported.

Therefore, we investigated the effects of AA on hair growth, via the proliferation of hDPCs and hair shaft elongation during human HF culture. We also explored the effect of AA on the expression of various hDPC growth and survival factors, as well as AA and EPAs ability to induce and prolong anagen in C57BL/6 mice.

Materials and Methods

Ethical considerations

This study's design was reviewed and approved by the institutional review board of Seoul National University Hospital (approval number C-1312-078-541), and all human volunteers provided their written informed consent. All experimental procedures that used human materials were conducted according to the principles described in the Declaration of Helsinki. All animal experiments were performed at the animal facility of Seoul National University Hospital, in accordance with the national and institutional guidelines for animal care.

Chemicals and reagents

We obtained lyophilized AA and EPA from Cayman Chemicals (Michigan, USA), and dissolved the AA and EPA in ethanol to create stock solutions of 100mM, 10mM, 5mM and 1mM; the stock solutions

were stored at −20°C until their use. For the animal experiments, 3% minoxidil (MNX) was obtained from Hyundai Pharm. Ind. Co. (Chonan, Korea).

Isolation of human HFs and DPCs

Skin biopsy specimens (1.5 × 1.0 cm scalp tissue samples from the occipital region) were obtained from healthy male volunteers (mean age 36.6±9.4 years) who did not have any current or prior scalp disease. The HFs were isolated under a dissecting stereomicroscope (Olympus, Tokyo, Japan), and the hDPCs were isolated from individual HFs that were morphologically determined to be in the anagen stage as previously described [16, 17].

Cell culture

As previously described [17, 18], the hDPCs were cultured at 37°C (5% CO_2) in Dulbecco's modified Eagle's medium (DMEM; Welgene, Daegu, Korea), which was supplemented with 10% fetal bovine serum (Welgene), 10 ng/mL of basic fibroblast growth factor (FGF; R&D

Systems, Minneapolis, MN, USA), and a 1×antibiotic /antimycotic solution (100 mg/mL streptomycin and 100 U/mL penicillin).

Viability and proliferation of hDPCs

The viability of the hDPCs was determined using the MTT (3-[4,5-dimethylthiazol-2-yl] -2,5-diphenyltetrazolium bromide) assay. In brief, hDPCs (1×10^4cells/well) were seeded into 96-well plates, cultured for 24h in serum-free DMEM, and then treated for 24h with the vehicle (ethanol that was diluted 1:1,000 in serum-free DMEM) or with AA (1-50μM [final concentration], diluted 1:1000 in serum-free DMEM). Next, 20μL of MTT solution (5mg/mL) was added to each well, and the plate was subsequently incubated for 3h at 37°C. The supernatant was then removed, and the formazan crystals were dissolved in 200mL of dimethylsulfoxide. Finally, the samples were incubated for 30min at room temperature, and were quantified by measuring their optical density at 570nm using a plate reader.

Human hair follicle organ culture

Human scalp HFs were isolated and cultured as previously described [19]. Each HF was cut at the level of the sebaceous duct, and was then cultured for 12 days at 37°C (5% CO_2)in Williams' E medium (Gibco BRL), which was supplemented with 10 ng/mL hydrocortisone, 10 μg/mL insulin, 2 mM L-glutamine, and the1×antibiotic/antimycotic solution (penicillin and streptomycin, Gibco BRL). In addition, AA was added to culture medium at final concentrations of 1μM, 2 μM, 5 μM, or 10μM. On every third day, the culture medium was changed and the elongation of the hair shaft in each HF was measured using a stereomicroscope (Olympus, Tokyo, Japan). A total of 400 HFs from 5 different volunteers (80 HFs per volunteer) were analyzed for each growth condition.

Immunohistochemistry and immunofluorescence staining

Immunohistochemistry and Immunofluorescence staining were performed using 5-μm paraffin sections of human HFs, as previously

described [20]. The antibodies that we used were anti-FGF-7 and anti-FGF-10 (Santa Cruz Biotechnology, Santa Cruz, CA, USA); anti-Ki67 (DAKO, Carpentaria, CA, USA) was used as a cell proliferation indicator, and a 4',6-diamidino-2-phenylindole (DAPI) mounting media kit (Vector Laboratories, Burlingame, CA, USA) was used to counterstain the nuclei.

Quantitative real-time polymerase chain reaction

Total RNA was isolated from the hDPCs using RNA iso Plus (Takara Bio Inc., Otsu, Shiga, Japan) and was treated with DNase I (Roche Pharmaceuticals, Welwyn Garden City, UK) to remove the genomic DNA. We then used 1–2 μg of total RNA for the cDNA synthesis reaction, which was performed using the First Strand cDNA Synthesis Kit (Fermentas, St. Leon-Rot, Germany), according to the manufacturer's instructions. To quantitatively estimate the mRNA expression, real-time polymerase chain reaction was performed using a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) with SYBR Premix Ex Taq (Takara Bio Inc.),

according to the manufacturers' instructions. The primer information is listed in Table 1; all experiments with SYBR Green were performed in triplicate and were independently repeated more than 3 times.

Western blotting

The total protein from the hDPCs was extracted using RIPA lysis buffer (Millipore, Billerica, MA, USA), according to the manufacturer's instructions. Proteins were separated using 10% and 15% sodium dodecyl sulfate polyacrylamide gel electrophoresis, and were transferred to a polyvinylidene fluoride membrane (Amersham, Buckinghamshire, UK) using a wet transfer system. The blotted membranes were incubated at 4°C with the appropriate antibodies: anti-total extracellular signal-related kinase (ERK), anti-phosphorylated ERK, anti-total protein kinase B (AKT), anti-phosphorylated AKT, anti-B-cell lymphoma 2 (Bcl2), anti-total cyclic AMP response element-binding protein (CREB), anti-phosphorylated CREB (Cell Signaling Technology, Beverly, MA, USA),

and anti-βactin (Santa Cruz Biotechnology). The membranes were then probed with anti-mouse, anti-rabbit, or anti-goatIgG-horseradish peroxidase conjugates (Santa Cruz Biotechnology) for 1 h at room temperature. Antibody-antigen complexes were then detected using the ECL system (Amersham Pharmacia Biotech, Little Chalfont, UK).

Induction of anagen in C57BL/6 mice

The methods that we used for the anagen induction assay have been previously described [21, 22]. In brief, we shaved the backs of 8-week-old female C57BL/6 mice (5 mice/group) that were in the telogen phase, and applied 200 μL of AA (2%), EPA (2%), AA plus EPA mixed by ratio of 1:1, 1:3, 3:1 (v/v) and the vehicle (negative control), or 3% MNX (positive control) on each day for a 4-week period. The back skin was observed daily, and a picture was taken each week. Back skin biopsies were performed on day 28, and the anagen hairs were confirmed via histological examination with hematoxylin and eosin staining.

In vivo model of depilation-induced hair regeneration

The back skin of 8-week-old female C57BL/6 mice in the telogen phase was depilated using wax, as described previously [23, 24]. After 10 days, all HFs in the depilated area had entered the anagen VI stage. From this time point onwards, we applied 200 μL of AA (2%), EPA(2%), AA plus EPA mixed by ratio of 1:1, 1:3, 3:1 (v/v) and the vehicle (negative control), or 3% MNX (positive control) each day for 10 days. Biopsies were performed on day 21, and the skin samples were stained with hematoxylin and eosin. To calculate the hair cycle score, we assigned the following arbitrary scores, as previously described [24]: HFs in anagen VI, 100; HFs in early catagen (catagen I–catagen III), 200; HFs in mid-catagen (catagen IV–catagen V), 300; and HFs in late catagen (catagen VI–VIII), 400. Five mice were used for each group, and 50 HFs per mouse were identified and graded on the biopsy specimens. The Image J program (National Institutes of Health (NIH), Bethesda, MD, USA) was used for quantitative analysis

Statistical analysis

Statistical significance was determined using Student's t test, and a paired t test was used to compare the HF culture results. All tests were two tailed, and differences with a P-value of < 0.05 were considered statistically significant.

Table 1. Primers for Human genes

Gene	Primer sequence
GAPDH (control gene)	
Forward	5'- ATT GTT GCC ATC AAT GAC CC-3'
Reverse	5'- AGT AGA GGC AGG GAT GAT GT-3'
FGF-7	
Forward	5'-TTTG TGG CAA TCA AAG GGG T- 3'
Reverse	5'- CCT CCG TTG TGT TGT CCA TTT AGC-3'
FGF-10	
Forward	5'-GTC TTC CGT CCC TGT CAC CT-3'
Reverse	5'-AAG GTG ATT GTA GCT CCG CA-3'
HGF	
Forward	5'-AGC AGT CCT CGT AAG GCA AA-3'
Reverse	5'-AAT CTG CCT GGA AAC ACC AC-3'

GAPDH, glyceraldehyde-3-phosphate dehydrogenase; FGF-7, Fibroblast growth factor 7; FGF-10, Fibroblast growth factor 10; HGF, Hepatocyte growth factor

.

RESULTS

1. AA and EPA induced anagen hair growth in telogen mouse skin

To investigate effect of AA and EPA on hair growth, we treated 8-week-old female C57BL/6 mice with AA , EPA (2%), AA plus EPA mixed by ratio of 1:1, 1:3, 3:1 (v/v) and the vehicle (negative control), or 3% MNX (positive control). In this context, the hair of C57BL/6 mice enters the telogen stage at postnatal week 7, which is the optimal time to examine anagen induction without endogenous hair growth [25]. In this experiment, we observed that 2% AA, AA plus EPA 3:1 or 3% MNX was able to produce a significantly higher level of hair growth and 2% EPA and AA plus EPA 1:1, 1:3 group were moderately accelerate hair growth at day 28, compared to the controls, as seen in Figure 1A. In addition, the change in back skin color change and hair re-growth percentage were significantly greater in mice that were treated with 2% AA, AA plus EPA 3:1 group or 3% MNX after 4 weeks, compared to the controls (Fig. 1B and C). These results indicate that AA and MNX are potent anagen inducers.

2. AA accelerated hair follicle growth and prolonged anagen hair growth

Next, we synchronized the dorsal areas of the mice to the anagen stage via depilation, as all HFs in the depilated skin areas enter the anagen VI stage at 10 days after depilation [24, 25]. At day 21, after 10 days of treatment (Fig. 2A), histological examination revealed an increase in the size, depth, and length of the HFs that were treated with AA (2%), EPA(2%), AA plus EPA mixed by ratio of 1:1, 1:3, 3:1 (v/v) and the vehicle (negative control), or 3% MNX (Fig 2B). In addition, the hair cycle scoring revealed markedly prolonged anagen hair growth in the HFs that were treated with 2 % AA and AA plus EPA 3:1 group. However, no significant difference in hair thickness was observed when we compared the 2% AA and vehicle groups (Fig 2C).

3. Effect of AA on DPC proliferation and hair shaft elongation in cultured human hair follicles

We used the MTT assay to evaluate the effect of AA on hDPCs' survival. In this experiment, we found that AA (1–5 μM) significantly enhanced viability of hDPCs compared to the vehicle-treated controls (Fig. 3A). However, the hDPCs' viability decreased at AA concentrations that were > 10 μM.

In addition, we examined hair shaft elongation by culturing human HFs that were treated with AA (1–10 μM) for 12 days. In this experiment, we found that 1μM, 2 μM, and 5 μM of AA significantly enhanced hair shaft elongation after 6, 9, and 12 days, compared to the vehicle-treated controls (Fig. 3B).

To analyze the proliferation of human HFs, we performed immunofluorescence staining for Ki-67 (a proliferation marker) after HFs were cultured with AA for 3 days. After counting the number of Ki67-positive cells among the follicular matrix keratinocytes (from the hair bulb), and normalizing this number using the number of DAPI positive cells, we found that treatment with AA (1–5 μM) significantly increased the number of Ki-67-positive keratinocytes (Fig.3C and D).

4. Expression of growth and survival factors in AA-treated DPCs

After treatment with AA (1μM, 2 μM, and 5 μM) for 24h, the levels of mRNA expression for FGF-7 and FGF-10 which are known to stimulate hair growth [26-28], exhibited a marked increase in a dose-dependent manner. In addition, the expression of hepatocyte growth factor (HGF) mRNA, another potent inducer of hair growth [29, 30], also significantly increased after AA treatment (Fig.4A), whereas the expression of insulin-like growth factor-1 and vascular endothelial growth factor mRNA were not affected (data not shown).

We performed immunohistochemical staining for FGF-7 and FGF-10, after the HFs were treated with AA for 3 days, and found that the expression of FGF-7 was increased in the cytoplasm of DP after treatment with 1μM, 2 μM, and 5 μM of AA. In addition, the expression of FGF-10 was increased in DP that were treated with 2 μM and 5 μM of AA (Fig. 4B).

In addition, we performed Western blotting to analyze the expression of various signaling molecules that are related to cell proliferation and survival, such as mitogen-activated protein kinase ERK, CREB,

AKT, and Bcl2 [23, 31, 32]. In this experiment, AA treatment enhanced phosphorylation of ERK, CREB, and AKT, and increased the expression of Bcl2 (Fig. 4C and D). These results indicate that AA affects intracellular signaling cascades and prevents cell death by increasing the phosphorylation of ERK and promoted cell survival up-regulation of AKT phosphorylation and Bcl2 expression.

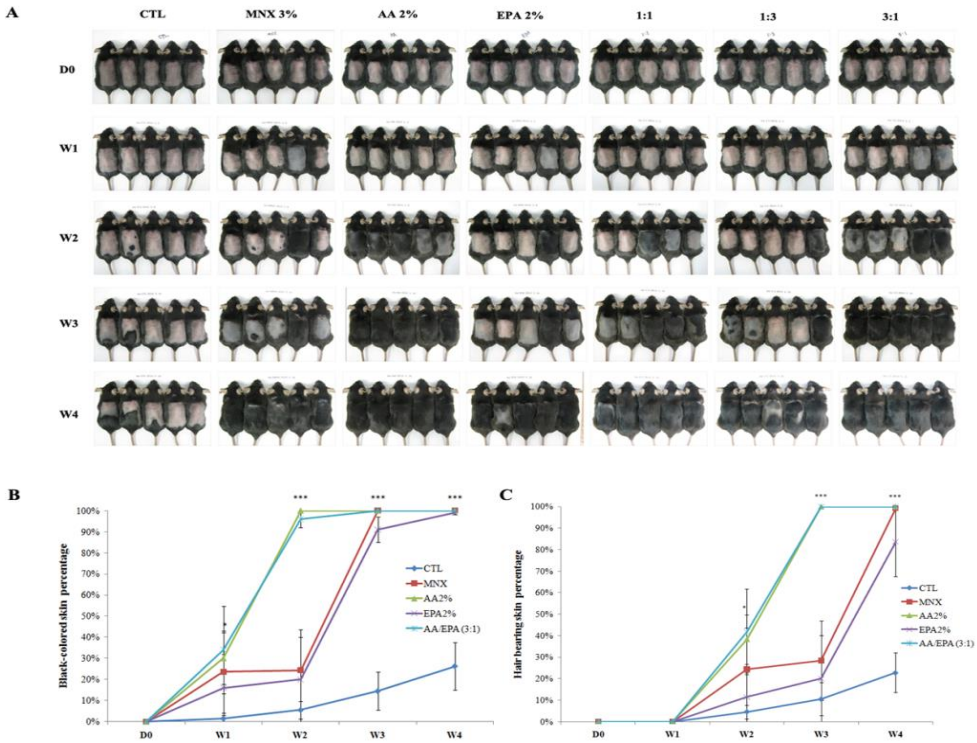

Fig 1. Enhanced anagen induction due to arachidonic acid (AA) treatment in 8-week-old female C57BL/6 mice. (A) After their backs were shaved, groups of 5 mice were treated with the vehicle, topical 3%minoxidil (positive control), or 2% AA, 2% EPA, AA plus EPA mixed by ratio of 1:1, 1:3, 3:1 (v/v) for 4 weeks.

(B) The amount of black colored skin according to week. (C) The amount of hair-bearing skin according to week. Data are expressed as mean ± standard error.* $P \leq 0.05$,*** $P \leq 0.001$ vs. the control group. CTL: control, MNX: 3% minoxidil, AA: arachidonic acid, EPA:

eicosapentaenoic acid D0: first day after the back was shaved, W1:1stweek, W2:2ndweek, W3:3rd week, W4:4thweek.

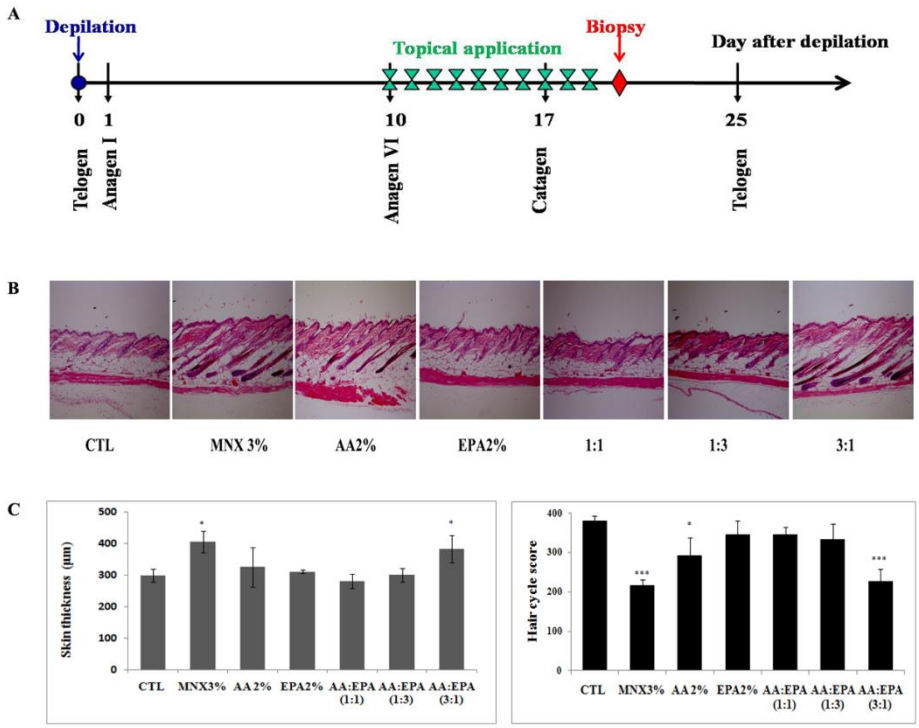

Fig. 2. Arachidonic acid and arachidonic acid plus eicosapentaenoic acid 3:1 group prolongs the anagen hair cycle.(A) The experiment design. After depilation via waxing on day 10, the back skin of C57BL/6 mice was treated with vehicle, topical 3%minoxidil, or 2% AA, 2% EPA, AA plus EPA mixed by ratio of 1:1, 1:3, 3:1 (v/v). (B) At day 20, skin samples were collected for histological analysis in hematoxylin and eosin-stained paraffin sections. (C) Calculating the hair cycle scores. For each mouse (5 mice per group), 50 hair

follicles (HFs) from each section were graded as follows: anagen VI = 100, early catagen = 200, mid catagen = 300, late catagen = 400. The score indicates the mean hair cycle stage for all HFs in group. Data are expressed as mean ±standard error. * $P \leq 0.05$, *** $P \leq 0.001$vs.the control group. CTL: control, MNX: 3% minoxidil, AA: arachidonic acid, EPA: eicosapentaenoic acid

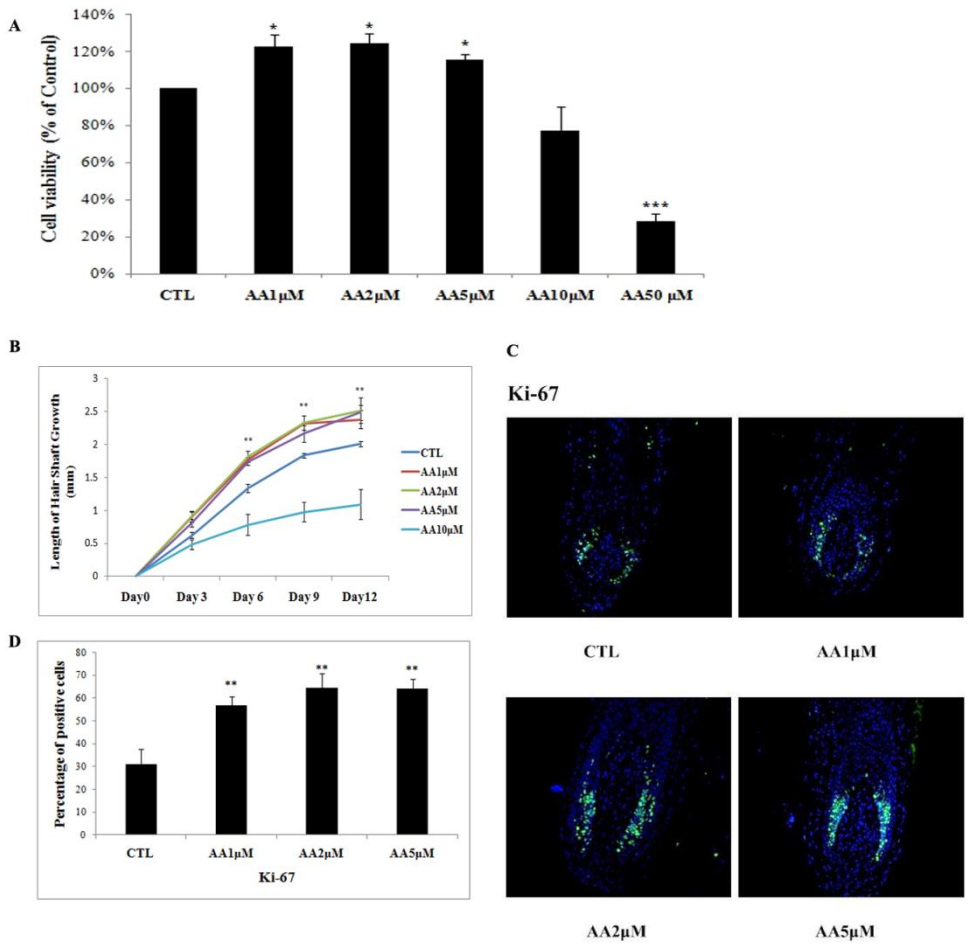

Fig. 3. Effect of arachidonic acid (AA) on hDPCs viability and hair shaft elongation. (A) Treatment of hDPCs with AA(1–5μM) resulted in significantly increased cell viability, as measured via the MTT assay. (B) AA-enhanced hair shaft elongation in *ex vivo* follicle culture. Human hair follicles (HFs) were treated with the vehicle or AA (1μM, 2 μM, 5 μM, or 10μM) for 12 days (n = 5); AA significantly enhanced

hair shaft elongation. (C and D) The proliferation of matrix keratinocytes increases in AA-treated HFs. Human HFs were cultured with the vehicle or AA (1μM, 2 μM, or 5 μM) for 3 days, and then we used immunofluorescence staining to examine proliferation in the hair matrix keratinocytes via Ki67 (proliferation, green fluorescence), and 4',6-diamidino-2-phenylindole (DAPI, blue fluorescence) to counter stain the nuclei. . Data are expressed as mean ± standard error. * $P \leq 0.05$, ** $P \leq 0.01$, ***$P \leq 0.001$ vs. the control group. CTL: control, AA: arachidonic acid, Original magnification: 200 × for Ki-67.

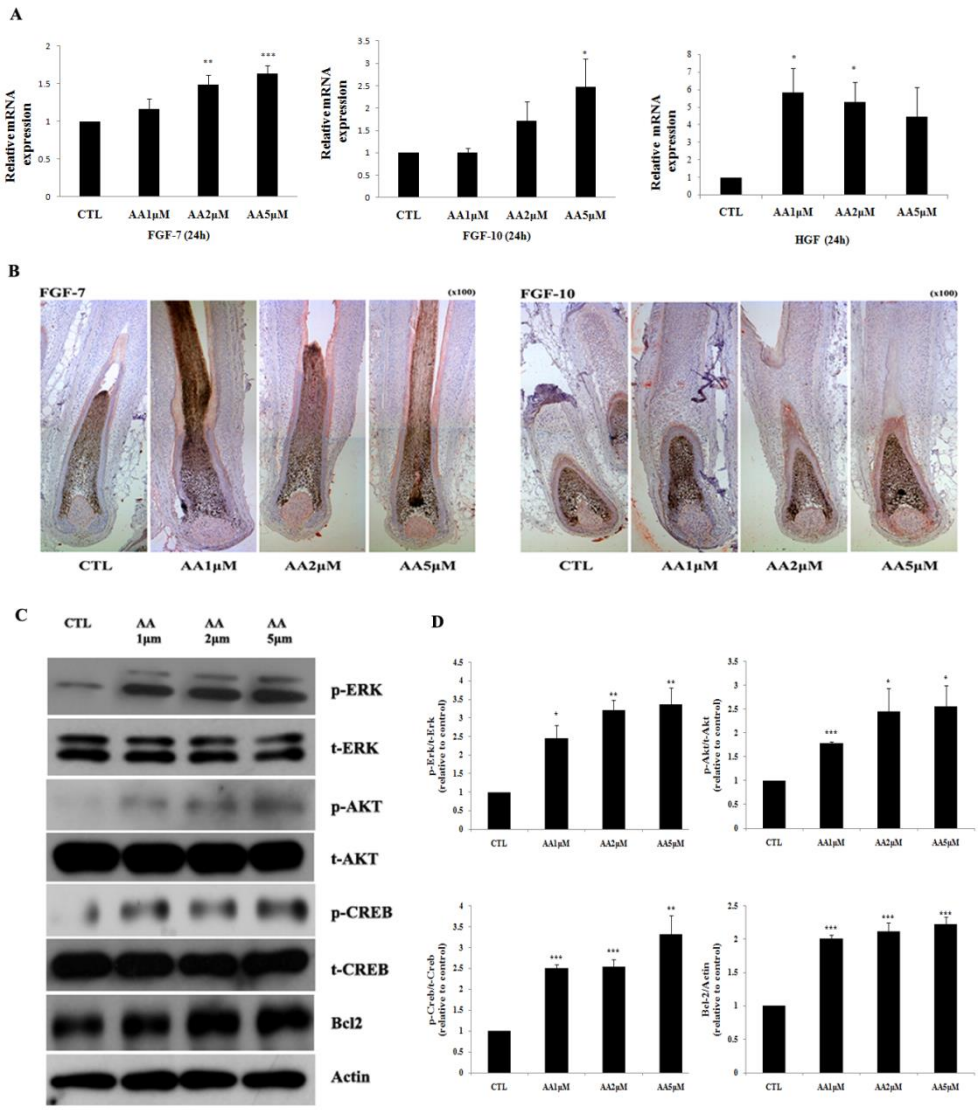

Fig. 4. Arachidonic acid (AA) increases the expression of growth and survival factor genes in human dermal papillary cells (hDPCs).

(A) The mRNA for fibroblast growth factors (FGF-7 and FGF -10) and hepatocyte growth factor growth factor (HGF) is elevated in hDPCs that were treated with AA (1μM, 2 μM, or 5 μM) for 24 h.
(B) Treatment with AA increases the expression of FGF-7 and FGF-10 in the cytoplasm of the dermal papilla (DP) and inner root sheath (IRS) at day 3, compared to the vehicle control. (C) We lysed hDPCs and analyzed the total protein content of the lysates via Western blotting, using primary antibodies for total-p42/44 ERK, phospho-p42/44 ERK, total-AKT, phospho-AKT, total-CREB, phospho-CREB, Bcl2, and β-actin.ERK: extracellular signal-regulated kinases, AKT: protein kinase B, CREB: cAMP response element-binding protein. (D) Quantitative analysis of western bands. Data are expressed as mean ±standard error. *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$ vs the control group. CTL: control, AA: arachidonic acid, FGF-7: fibroblast growth factor7, FGF-10: fibroblast growth factor10, HGF: hepatocyte growth factor. Original magnification: 100× for FGF-7 and FGF-10.

DISCUSSION

In the anagen hair cycle induction test we confirmed that AA treatment in C57BL/6 mice significantly affected anagen induction. Furthermore anagen hair prolongation test AA plus EPA 3:1 treatment kept hair follicles in their anagen stage longer as compared other groups. Which was indicated the importance of AA and EPA ratio in hair growth.

Epithelial-mesenchymal interactions play an important role in the organogenesis of many tissues, including HFs. In this context, the DP contains a population of mesenchymal cells that regulate hair matrix keratinocytes via various molecular signals, including growth factors [33]. In the present study, we found that AA increased the expression of FGF-7, FGF-10, and HGF. Both FGF-7 and FGF-10 stimulate cell proliferation and survival in a paracrine manner, and their expression peaks during anagen [26, 27, 34]. Furthermore, HGF regulates the function of various organs, by acting as a paracrine factor that is secreted by mesenchymal-derived cells and acts on neighboring epithelial or endothelial cells, as demonstrated

via *in vitro* studies of human HFs and mouse vibrissae [29, 30]. We were able to confirm this activity in our *ex vivo* culture of HFs, as 3 days of culture with AA increased the proliferation of human hair matrix keratinocytes and the expression of FGF-7 and FGF-10. Therefore, our results suggest that, in cultured hDPCs and *ex vivo* cultured HFs, treatment with AA affected the signal transduction cascades that mediate cell proliferation via expression of FGF-7, FGF-10, and HGF.

Furthermore, there would be direct stimulatory effect of AA on matrix keratinocytes or epithelial cells of outer root sheath (ORS). Several studies have investigated the effect of AA and its metabolites on skin keratinocytes. It was found that prostaglandin E receptor EP2 and EP3 subtypes have real effects on the rate of proliferation of keratinocytes [35]. Moreover, it was demonstrated ORS keratinocytes also expressed EP2, EP4, TP and to a lesser extent FP and DP_2 receptors in anagen human hair follicles [36].

In the Western blot analysis of hDPCs, we observed that AA treatment lead to phosphorylation of various transcription factors (ERK, CREB, and AKT) and increased the expression of Bcl2; these

factors are known to promote survival and prevent cell death [23, 37, 38]. Therefore, it appears that AA affects a variety of cellular functions, by triggering multiple intracellular signal transduction pathways. In our study, it would be uncertain how exogenously applied AA exert these effects. Some studies showed that the AA converted directly by COX, P450 and LOX enzymes and their metabolites play a role in the regulation of growth and motility in various cell types and utilization is depends on added AA concentration and cell type [39, 40]. Prostaglandin, one of the final metabolites of AA showed hair growth modulation with different analogs. PGE_2 analogs have been investigated as agents against radiation or doxorubicin induced alopecia in a murine model of hair injury. Both systemic and topical application of a PGE_2 analog resulted in a significant degree of protection against radiation or doxorubicin induced alopecia [41-43]. In another study viprostol, another PGE_2 analog administration led to human scalp hair growth providing an argument that the E series prostaglandins may have an effect on hair growth although to a lesser extent than the F series [44]. Recently some studies have demonstrated that hair growth can

be promoted using latanoprost and isopropyl unoprostone, which are analogues of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, the final metabolite of AA) [15], although the underlying mechanism of latanoprost-associated hair growth is not clearly understood.

In conclusion, our results demonstrate that AA treatment increases the proliferation of DPCs, promotes the elongation of human hair shafts, and promotes hair growth in a mouse model. These results may be related to the ability of AA to increase the expression of proliferation factors in HFs. Therefore, AA treatment is a potential candidate for the promotion of hair growth.

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국문초록

모발의 모습은 사람의 첫인상을 결정짓는 중요한 요소로, 탈모는 미용적 문제를 포함하여 삶의 질적인 측면에서도 중요한 문제가 될 수 있다. 발모제를 개발하기 위하여 다양한 연구가 진행되고 있으나 현재까지는 finasteride 나 dutasteride 와 같은 5α 환원효소억제제와 minoxidil 외에는 효과적인 치료방법이 부족한 형편이다. Polyunsaturated fatty acid (PUFA)는 기본 구조(backbone)를 비교해 볼 때 C-C double bond 보다 많은 long-chain 의 지방산으로 구성되어 있다. 오래 전부터 몇몇 나라에서는 전통치료요법으로써 고도불포화 지방산이 많이 함유된 식물 혹은 동물기름을 사용하여 탈모의 치료에 사용하기도 하였다. Arachidonic acid (AA) 와 Eicosapentaenoic acid (EPA)는 각각 오메가-6 와 오메가-3 로 불리고 있으며 이러한 지방산은 membrane phospholipid 의 변화를 통해 혹은 eicosanoids 와 같은 신호전달물질을 생산함으로써 영향을 미친다. 본 연구에서는 고도불포화 지방산의 대표적 물질인 arachidonic acid (AA) 및 eicosapentaenoic acid (EPA)가 모발 성장에 미치는 영향 및 기전을 밝히고자, C57BL/6 마우스를 이용하여 AA 와 EPA 가 anagen induction 과 anagen elongation 에 어떠한 영향을 미치는지 연구하였다. 또한 사람의 모낭 유두세포 (dermal papilla cells)와 *ex vivo* 즉, 모낭 기관을 배양하여 AA 와

EPA 의 모발성장에 대한 영향을 보기 위해,2% AA 와 2% EPA, 그리고 AA 와 EPA 를 1:1, 1:3, 3:1 으로 혼합한 물질을 C57BL/6 마우스 성장기 유도실험에 적용한 결과, 모든 물질들이 새로운 털을 재생하는데 있어서 통계학적으로 유의성 있는 효과를 보여주었다. 그리고 2% AA 와 AA 와 EPA 를 3:1 로 섞은 혼합물은 휴지기 상태에서 마우스의 털을 뽑아 anagen 을 유도한 마우스에서 성장기 기간을 연장시키는 것을 관찰할 수 있었다. 또한 AA 는 모낭 기관의 배양 (ex vivo) 에서도 모낭의 길이를 증가시키는 것을 관찰 할 수 있었다. 사람의 모낭 유두세포 (hDPCs)에 AA 를 저농도로 처리했을 때 제 7 형 및 제 10 형 섬유아세포성장인자의 mRNA 발현이 증가하며 인산화된 ERK 의 단백질도 증가한 것을 관찰할 수 있었다. 이러한 결과는 PUFA 의 대표적 물질로써 알려진 AA 또는 AA 와 EPA 혼합물들이 모발의 성장을 촉진하는 역할을 할 수 있음을 시사하는 소견들로, 해당물질들이 탈모증의 치료에 상당한 효과를 보일 가능성을 기대할 수 있을 것으로 판단된다.

주요어 : 모발 성장, 불포화 지방산, 아라키도닉산, 에이코사펜타에노산, 모발 주기, 모유두 세포

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