Investigation of the topical application of procyanidin oligomers from apples to identify their potential use as a hair-growing agent

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Summary

Background Procyanidins are a family of condensed tannins, which have been shown to possess hair-growing activity in both the *in vitro* and *in vivo* murine models.
Aims We report a 12-month clinical study aimed at treating male pattern baldness by external application of 0.7% apple procyanidin oligomers.
Patients/methods A double-blind clinical test involving a total of 43 subjects was performed. Twenty-one men in the procyanidin group and 22 men in the placebo control group were subjected to analysis. In the first 6 months, we compared the procyanidin and the placebo groups to assess the medicinal effects of procyanidin oligomers. The application time of the procyanidin group was subsequently extended to 12 months, and the time course of its remedial value was examined.
Results The increase in total number of hairs in a designated scalp area of the procyanidin group subjects after the 6-month trial was significantly greater than that of the placebo control group subjects (procyanidin, 3.3 ± 13.0 (mean $\pm$ SD)/0.50 cm^2; placebo, -3.6 ± 8.1/0.50 cm^2; $P < 0.001$, two-sample *t*-test). Time course-dependent improvement in hair density was observed in the procyanidin subjects. No adverse side effects were observed in any of the subjects. Procyanidin therapy thus shows potential hair-growing activity.

Keywords: external application, male pattern baldness, *Malus pumila*, procyanidin oligomers, scalp

Introduction

Proanthocyanidins are a species of polyphenol known to possess a variety of physiological activities, including radical scavenging activity,[1] antioxidative properties,[2] antifungal effects,[3] antiallergic activity all *in vitro*,[4] and antihypertensive activity *in vivo*,[5] and have been used as a treatment for capillary stabilization.[6] We have reported that procyanidin oligomers such as procyanidin B-2 (Fig. 1) possess growth-promoting activity in murine hair epithelial cells at a very high rate of 300% relative to controls, and have also demonstrated that procyanidin oligomers stimulate anagen induction in the *in vivo* murine model.[7] We isolated highly purified procyanidin oligomers, in particular procyanidin B-1, procyanidin B-2, and procyanidin C-1 from apples on an industrial scale[8] and subjected them to a series of toxicological studies. Our results confirmed the safety of topical application of procyanidin oligomers to human skin. We report here the results of a volunteer test focusing on the topical

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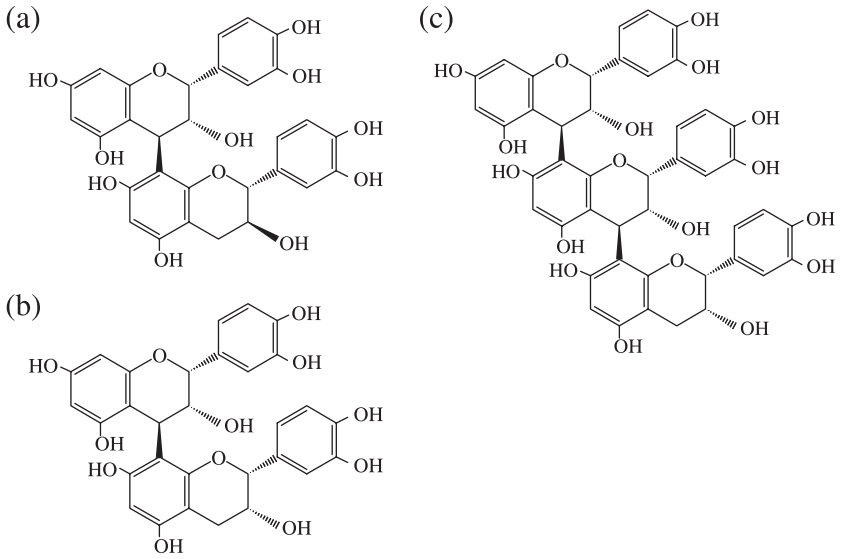

Figure 1 Structures of procyanidin oligomers. (a), Procyanidin B-1 [epicatechin-(4β(→)8)-catechin]; (b), procyanidin B-2 [epicatechin-(4β(→)8)-epicatechin]; (c), procyanidin C-1 [epicatechin-(4β(→)8)-epicatechin-(4β(→)8)-epicatechin].

application of procyanidin oligomers of high purity to test whether or not these procyanidin oligomers exhibited a curative effect on male pattern baldness or a delaying effect on the development of male pattern baldness; thus, changes in hair density was assessed.

Materials and methods

Subjects

Volunteer subjects (27–58 years old, male, in good health) were chosen by pre-examination and randomly allotted to the procyanidin and placebo groups. The following criteria were met by all subjects: they showed male pattern baldness on the scalp, had no dermatological disorders other than male pattern baldness on the scalp, had no other diseases, and were not undergoing any medical treatment. The exclusion criterion was the use by the subjects of hair-growing agents within the previous 6 months. The pattern of baldness was classified according to the Ogata scale,[9] specific to Japanese males (Table 1).

Table 1 Profile of the subjects.

	Baldness patterns (Ogata scale*)			
Group	Type I	Type II	Type III	Type IV
Placebo	2	11	0	9
Procyanidins	2	8	0	11

*Normal: ; type I: ; type II: ; type III: ; type IV: .

Study schedule

The subjects completed the outcome measures to provide baseline data and were given their first supply of test agents. The procyanidin group subjects (25 men) were treated with 0.7% (w/w) procyanidin agent, and the placebo control group (24 men) was treated with vehicle alone. The test agent (about 2 mL per dose) was applied to the subjects' affected scalp area twice daily, giving a daily dose of 18.7 mg of procyanidin oligomers. No use of other hair care products, apart from ordinary shampoos and rinses, was permitted during the clinical trial. The tests were performed in a double-blind fashion. In the first 6 months, we compared the procyanidin group and the placebo group to assess any medicinal effects of procyanidin oligomers. After completion of the 6-month period of twice-daily application of these agents, they returned for their visit to complete the outcome measures. The hair-growing effects were evaluated according to the macrophotographically recorded changes in the number of hairs in the designated scalp area. The application time of the procyanidin group was subsequently extended to 12 months, and the time course of the remedial value was examined. After completion of the 12-month period of twice-daily application of the agent, they returned for their visit to complete the testing of outcome. The hair-growing effects were evaluated by the macrophotographically recorded changes in the number of hairs in the designated scalp area.

Determination of change in hair density

Before the test, 6 months later, and 12 months later, hairs at a predetermined site (a round area 1 cm in

diameter) were clipped with small straight surgical scissors.[10] The site was selected from the outskirts of the affected area on the vertex of each subject using a plastic template connected by a strut to the frame of a pair of eyeglasses, for the purpose of precisely identifying specific scalp area. The hair-cutting sites were photographed using a camera (OM-4 Ti, Olympus Optical Co., Japan) fitted with a macro lens (Zuiko Auto-macro 38 mm, F2.8) and extension tube 25. The hairs in the photograph of this specific area (a round area 0.8 cm in diameter; area = 0.50 cm^2) at the predetermined site were counted three times each by three independent investigators.

Statistical analysis

The differences in total hair increase after the 6-month trial between the placebo and treatment groups were analyzed using the two-sample *t*-test. The increase in the total number of hairs after the 12-month trial period was compared with the baseline and a paired *t*-test was used for the analysis. All differences were considered significant at a level of $P < 0.05$.

Diagnosis by dermatologist

During the course of the 12-month test period, all subjects underwent clinical diagnosis by one of the authors, a dermatologist, who examined the subjects for any adverse dermatological reactions such as inflammation, erythema, or eczema.

Product characteristics

Purified procyanidin oligomers were obtained from unripe apples (*Malus pumila* Miller var. domestica Schneider, Fuji variety) according to the method described in a previous report.[8] The total procyanidin content was colorimetrically measured by a method described by Porter *et al.*[11] and was calculated to be 83.6% (w/w) using procyanidin B-2 as a standard. The product contained 7.3% (w/w) procyanidin B-1, 26.2% (w/w) procyanidin B-2, and 7.7% (w/w) procyanidin C-1 as the major components; other oligomeric procyanidins were also present about 40% (w/w).

Preparation of hair tonic for the clinical trial

The test agent was prepared by dissolving 0.7% (w/w) of procyanidin oligomers from apples in conventional basal solvent including 70% (w/w) of ethanol, 3% (w/w) of 1,3-butylene glycol, 0.15% (w/w) of *N*-acetylglutamine isostearyl ester, 0.067% (w/w) of citrate-sodium citrate buffer, 0.05% (w/w) of sodium bisulfite, and purified water (the remainder). Vehicle without procyanidin oligomers was used as the placebo control.

Ethical approval

Individual subjects agreed to an informed consent contract. The contract confirmed their willingness to participate in the test, their freedom to drop out at any time, and their willingness to use the agent under the administration of the doctor. The confidentiality of each participant's information was also safeguarded under this contract.

Results and discussion

Withdrawals

Data were available from 43 of the 49 patients who began the trial. Four participants in the procyanidin group and two in the placebo group dropped out for personal reasons. Ultimately, 21 men in the procyanidin group and 22 men in the placebo group were subjected to analysis. No other person dropped out for any reason.

Adverse effects

Dermatologic diagnosis revealed that no adverse side effects were caused by the agent, i.e., no inflammation, irritation, or allergic reactions of the scalp were observed in any of the subjects. Furthermore, no subjects complained of itching, pain, dryness, or scaling of the scalp.

Changes in hair density

In the procyanidin group, the increase in number of total hairs in the designated scalp area (a circle 0.8 cm in diameter = 0.50 cm^2) after the 6-month trial was 3.3 ± 13.0 (mean ± SD)/0.50 cm^2, whereas in the placebo control group, the increase in number of total hairs was -3.6 ± 8.1 (mean ± SD)/0.50 cm^2. It is calculated that the increased number of total hairs in the designated scalp area of the procyanidin group subjects after the 6-month trial was significantly greater than that of the placebo control group subjects ($P < 0.001$, two-sample *t*-test) (Fig. 2). The total number of hairs in the designated scalp area after the 12-month procyanidin treatment significantly increased over the baseline value measured at the start of the trial (11.5 ± 16.5 (mean ± SD)/0.50 cm^2; $P < 0.005$, paired *t*-test) (Fig. 2). These results show a time-course-dependent improvement in hair density in the procyanidin group subjects.

Human clinical trials of topical procyanidins

As the procyanidin oligomers were revealed to possess the highest hair growth-inducing potential of the various proanthocyanidin molecules we had investigated using the murine model,[7] we considered investigating the use of procyanidin oligomers to treat male pattern baldness. We obtained procyanidin oligomers of high purity on an industrial scale from apples for practical use, and conducted a 12-month clinical trial.

We observed a total increase of 23 hairs/cm^2 after 12 months of procyanidin treatment. The same effects have been reported with minoxidil[12] and finasteride[13] therapy for male pattern baldness. With the 2% minoxidil treatment, a total increase of 250 hairs/5.1 cm^2 (calculated as a total increase of 49 hairs/cm^2) after 12 months of therapy was reported.[12] With finasteride treatment (1 mg/day, oral administration), a total increase of 86 hairs/5.1 cm^2 (calculated as a total increase of 16.9 hairs/cm^2) after 12 months of therapy was reported.[13] The level of efficacy of our 0.7% procyanidin oligomer preparation was thus considered to compare favorably with both minoxidil and finasteride therapy.

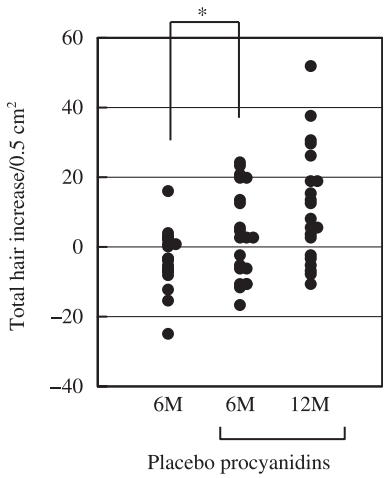

Figure 2 Change in hair density. The increased number of total hairs in the designated scalp area after the 6-month treatment of placebo control, 6-month treatment of procyanidin agent, and 12-month treatment of procyanidin agent over the baseline value are shown. The increase in total number of hairs in a designated scalp area of the procyanidin group subjects after the 6-month trial was significantly greater than that of the placebo control group subjects (*$P < 0.001$, two-sample t-test). The total number of hairs in the designated scalp area after the 12-month procyanidin treatment significantly increased over the baseline value measured at the start of the trial ($P < 0.005$, paired t-test).

Assumed mechanisms of action

TGF-β has recently been hypothesized to be a catagen-inducing factor,[14,15] and lipid peroxidation[16] and inflammation[17] have been observed to be aggravating factors. As the hair-growing mechanisms of procyanidins, TGF-β-related mechanisms,[18] or mechanisms affected by antioxidation and anti-inflammation are speculated. It appears that the activity of procyanidin oligomers may depend on more than one of their numerous physiological functions.

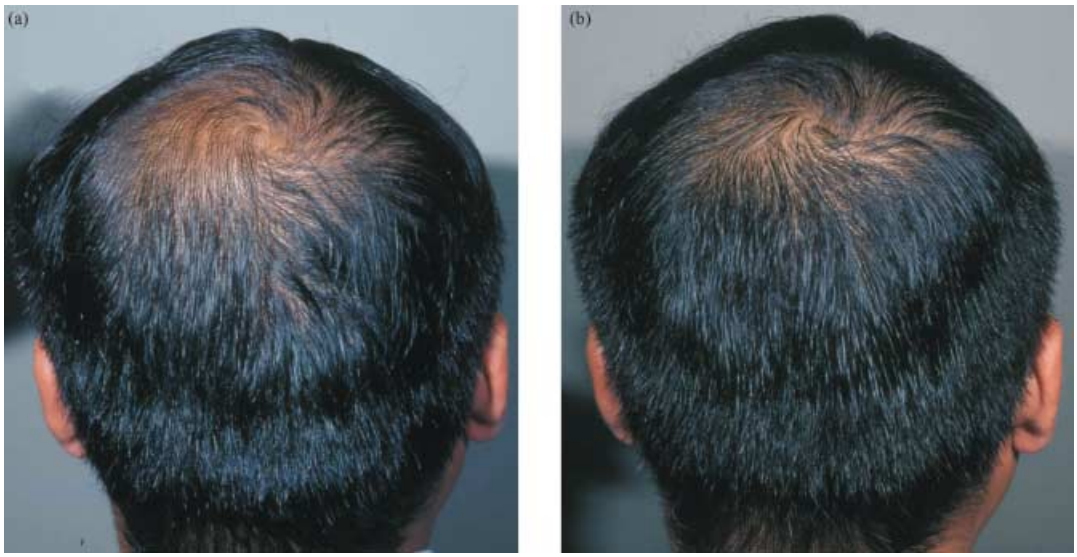

Figure 3 Clinical photographs of a subject before (a) and after (b) 12-month treatment of procyanidin agent.

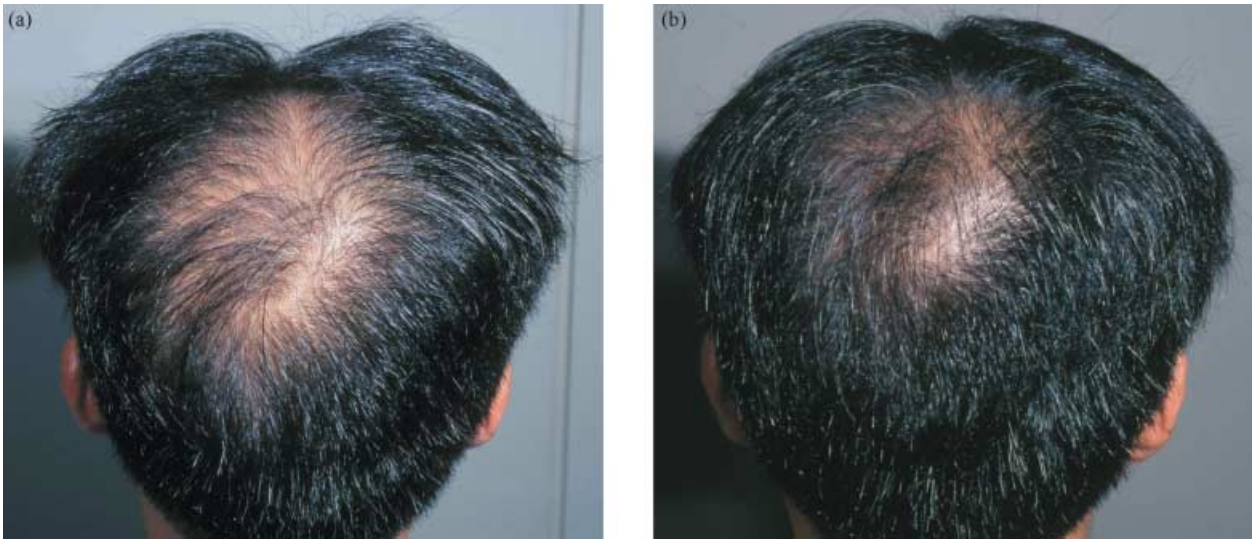

Figure 4 Clinical photographs of a subject before (a) and after (b) 12-month treatment of procyanidin agent.

Effects on hair growth

This investigation provides evidence that procyanidins may impede the development of male pattern baldness. A number of the subjects showed cosmetically satisfactory changes (Figs 3 and 4). This study suggests that the use of procyanidin oligomers to treat male pattern baldness merits further investigation.

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