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ORIGINAL ARTICLE

Adenosine increases anagen hair growth and thick hairs in Japanese women with female pattern hair loss: A pilot, double-blind, randomized, placebo-controlled trial

Hajimu OURA,[1,⋆] Masato IINO,[2,⋆] Yosuke NAKAZAWA,[2] Masahiro TAJIMA,[2] Ritsuro IDETA,[2] Yutaka NAKAYA,[3] Seiji ARASE,[1] Jiro KISHIMOTO[2]

[1]*Department of Dermatology, School of Medicine, University of Tokushima, Tokushima,* [2]*Shiseido Research Center, Yokohama-shi, and* [3]*Department of Nutrition and Metabolism, Institute of Health Biosciences, University of Tokushima Graduate School, Tokushima, Japan*

ABSTRACT

Adenosine upregulates the expression of vascular endothelial growth factor and fibroblast growth factor-7 in cultured dermal papilla cells. It has been shown that, in Japanese men, adenosine improves androgenetic alopecia due to the thickening of thin hair due to hair follicle miniaturization. To investigate the efficacy and safety of adenosine treatment to improve hair loss in women, 30 Japanese women with female pattern hair loss were recruited for this double-blind, randomized, placebo-controlled study. Volunteers used either 0.75% adenosine lotion or a placebo lotion topically twice daily for 12 months. Efficacy was evaluated by dermatologists and by investigators and in phototrichograms. As a result, adenosine was significantly superior to the placebo according to assessments by dermatologists and investigators and by self-assessments. Adenosine significantly increased the anagen hair growth rate and the thick hair rate. No side-effects were encountered during the trial. Adenosine improved hair loss in Japanese women by stimulating hair growth and by thickening hair shafts. Adenosine is useful for treating female pattern hair loss in women as well as androgenetic alopecia in men.

Key words: adenosine, female pattern hair loss, hair appearance, hair growth rate, thick hair rate.

INTRODUCTION

Male pattern hair loss, also known as androgenetic alopecia (AGA), usually appears gradually with miniaturization of hair follicles and successive hair shedding and is androgen-dependent.[1] In contrast, female pattern hair loss (FPHL) is typically characterized by a reduction in hair density in the central scalp area, and by the miniaturization of hair follicles.[2] Ludwig previously demonstrated that AGA in women differs from hair loss in men,[3] and the roles of androgens in hair loss in women remain unclear.

Topical administration of minoxidil has been shown to be effective in treating AGA in men by thickening hair shafts,[4] and for FPHL by increasing hair weight and number.[5] Minoxidil induces hair growth by increasing the proliferation of follicular epithelial cells via the stimulation of blood microcapillary circulation by opening potassium channels.[6] It was proposed that an adenosine-mediated signal transduction pathway contributes to hair growth based on evidence that the activation of adenosine receptors regulates minoxidil-induced production of vascular endothelial growth factor (VEGF) in cultured dermal papilla cells.[7] A randomized clinical trial on 102 Japanese men with AGA evaluated adenosine as a hair growth promoter and showed that a topical adenosine lotion was significantly superior to a placebo in thickening hair shafts (full report is under submission).[8]

Correspondence: Jiro Kishimoto, Ph.D., Shiseido Research Center, 2-2-1 Hayabuchi, Tsuduki-ku, Yokohama City 224-8558, Japan. Email: masato.iino@to.shiseido.co.jp

⋆These authors contributed equally to this work.

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In this study, we investigated the efficacy and safety of topical adenosine use as a hair growth promoting agent against hair loss in Japanese women.

METHODS

Subjects and treatment

A double-blind, randomized, placebo-controlled study was conducted on 30 women aged 22–53 years (mean, 38.9 years) for 12 months. Subjects were clinically diagnosed with FPHL over stage 1.5 of hair loss,[9] with no evidence of internal systemic illness. Institutional review boards approved the study protocol and the informed consent form. All volunteers received a full explanation of this trial and signed a written informed consent prior to the trial. Randomization was carried out to divide the volunteers into two groups statistically equal in age and degree of hair loss.

Subjects applied approximately 3 mL of a lotion containing 0.75% adenosine or a lotion without adenosine (placebo) topically, twice daily for 12 months. Data were obtained at the beginning of the trial and at 6 and 12 months thereafter.

Methods

Dermatologists performed a physical examination of each subject, especially checking for signs of dermatitis on the scalp, such as erythema, swelling, eczema, seborrhea or scaling, and estimated the severity of hair loss. All diagnostic information was recorded on each subject's case card.

Efficacy evaluation

Dermatologist assessments

Dermatologists examined each subject visually and rated the severity of hair loss by comparing with standard photographs of hair loss severity, using a 6-point scale from stage 1 (no hair loss) to stage 6 (detectable hair loss). These six standard photographs were selected based on the distinctness of the levels of hair loss in each photo.[9] To evaluate rate of hair loss of each subject accurately, we also set intermediate values such as 1.5, 2.5, 3.5, 4.5 and 5.5 between stages 1 and 6. Changes in rating between baseline and 6 or 12 months represented improvement of hair loss as follows: if the range of rating change was 1 or over (toward the minus direction), the assessment was "improvement", and between 0.5 to 1.0, the assessment was "slight improvement". When the change was worse (towards the plus direction) with a range of rating change of 1 or over, the assessment was "decline", and between 0.5 to 1.0, the assessment was "slight decline". When the range of change was less than 0.5, the assessment was "no change".

Investigator assessments

Scalp hairs of subjects were combed along a coronal midline before photography. Top views of scalps were photographed using a digital camera (EOS-1, Canon Corp, Tokyo, Japan). Three well-trained investigators rated each printed photograph using the same scales described in the dermatologist assessments above. The mean ratings of the three investigators represented the severity of hair loss. Changes in rating between baseline and 6 or 12 months represented improvement of hair loss as follows: If the range of rating change was 0.5 or over (toward the minus direction), the assessment was "improvement", and less than 0.5 to 1.0, the assessment was "slight improvement". When the change was worse (toward the plus direction), the range of rating change of 0.5 or over, the assessment was "decline", and less than 0.5, the assessment was "slight decline". When the range did not change at all, the assessment was "no change".

Phototrichograms

Hairs in an approximate 6 mm $\times$ 6 mm square located 40 mm from the coronal midline on the scalp to the ear, were clipped using ophthalmologic scissors.[10] The clipped area was digitally imaged using a video microscope (VHX-100, Keyence, Osaka, Japan) at a $\times$30 magnification at the time of clipping (day 0) and 2 days later (day 2). The clipped area was quantified for pixel numbers using image processing software (Mac Scope, Mitani, Fukui, Japan). Hair numbers in each area were counted, and hair density was calculated as the number of hair shafts/cm^2. Hair growth rates were estimated from images at day 0 and at day 2. Anagen hairs are defined as hairs with a growth rate of more than 0.2 mm/day. Hair thickness was classified into three groups: (i) thick hairs

Table 1. Assessment of hair characteristics following treatment

		Month 6			Month 12		
	Change of rating of hair appearance	Adenosine (n = 13)	Placebo (n = 14)	P-value	Adenosine (n = 13)	Placebo (n = 14)	P-value
Dermatologist assessments	–1.0 ("improvement")	0 (0%)	0 (0%)		4 (31%)	2 (14%)	
	–0.5 ("slight improvement")	5 (39%)	3 (21%)	0.3419*	7 (54%)	3 (21%)	0.0238*
	0 ("no change")	8 (62%)	11 (79%)		2 (15%)	9 (64%)	
	0.5 ("slight decline")	0 (0%)	0 (0%)		0 (0%)	0 (0%)	
	1.0 ("decline")	0 (0%)	0 (0%)		0 (0%)	0 (0%)	
	–1.0 and –0.5	5 (39%)	3 (21%)	0.4197*	11 (85%)	5 (36%)	0.0183†
	0 and above	8 (62%)	11 (79%)		2 (15%)	9 (64%)	
Investigator assessments	–0.5 and below ("improvement")	2 (15%)	1 (7%)		3 (23%)	0 (0%)	
	Above –0.5 and less than 0 ("slight improvement")	1 (8%)	3 (21%)		5 (39%)	5 (36%)	
	0 ("no change")	8 (62%)	5 (36%)	0.5149*	4 (31%)	5 (36%)	0.0337*
	Above 0 and less than 0.5 ("slight decline")	2 (8%)	5 (36%)		1 (8%)	5 (36%)	
	0.5 and above ("decline")	0 (0%)	0 (0%)		0 (0%)	1 (7%)	
	Minus	3 (23%)	4 (29%)	1.0000†	8 (62%)	5 (36%)	0.2568†
	Null and plus	10 (77%)	10 (71%)		5 (39%)	9 (64%)	

*Mann–Whitney U-test. †Fisher's exact probability test.

(>80 μm in diameter); (ii) thin hairs (<40 μm in diameter);[11] and (iii) others.

Subject self-assessments

At 6 and 12 months, subjects self-assessed the condition of their scalp hair compared to the baseline using a questionnaire based on a previous report.[12]

Statistical analysis

Comparisons between the adenosine and placebo groups were statistically analyzed using Mann–Whitney U-tests and/or Fisher's exact probability tests for dermatologist, investigator and self-assessments. Measurements in the phototrichograms at months 6 and 12 were compared with baseline data using a paired two-tailed Student's t-test. Changes between groups were analyzed using a non-paired two-tailed Student's t-test. In all cases, tests at $P < 0.05$ were considered to be statistically significant. The dermatologist assessments were used as the primary end-point.

RESULTS

Thirty women with FPHL were enrolled in the trial, and 13 of 15 and 14 of 15 volunteers completed the 12-month study for the adenosine and placebo groups, respectively. In each group, one subject withdrew from the study before using the test lotion. One adenosine-treated subject could not continue the trial due to personal reasons at month 12. Therefore, 27 and 28 subjects were included in the study population for the efficacy evaluation and for the safety evaluation, respectively. No significant differences ($P > 0.2$) in baseline characteristics were observed between the two groups.

Adenosine-treated subjects showed significant improvement according to the dermatologist assessments (primary end-point) at month 12 (Table 1) compared to the placebo-treated subjects. Eleven of 13 (85%) subjects in the adenosine-treated group were assessed as "improvement" or "slight improvement" while only five of 14 (36%) subjects in the placebo-treatment were assessed similarly at month 12. The investigator assessments by photographs were consistent with the dermatologist assessments (Table 1).

Phototrichogram results were as follows. Anagen hair ratio, thin hair ratio and hair density did not change significantly between the groups at any time point (Fig. 1a,d,e, respectively). However, the anagen hair growth rate of the adenosine-treated group significantly increased both at month 6 and at

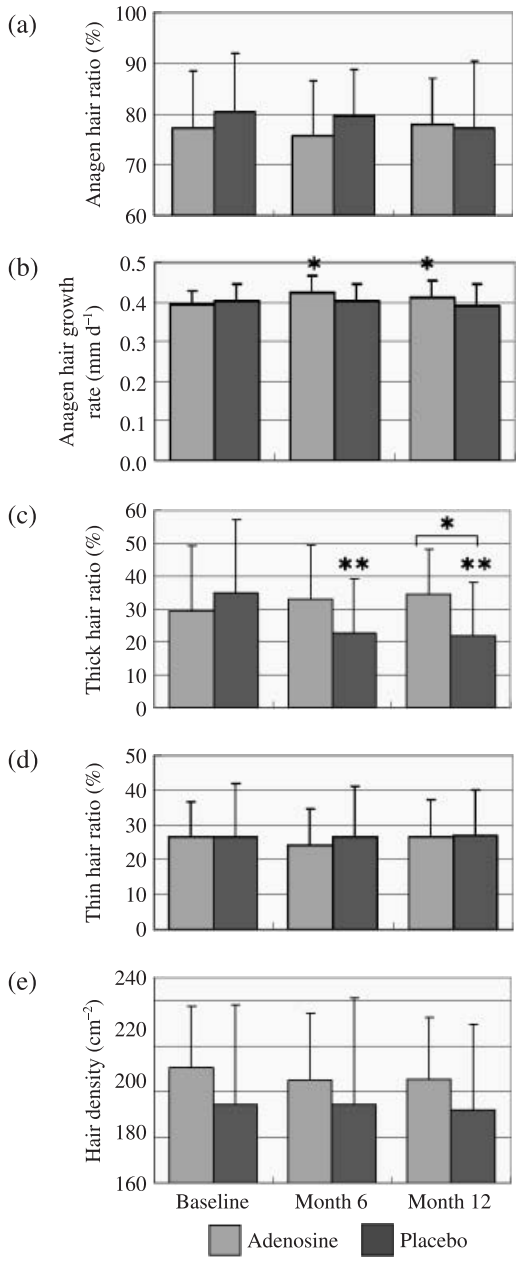

Figure 1. Anagen hair ratio, anagen hair growth rate, thick and thin hair ratios, and hair density. Thick hairs and thin hairs are defined as over 80 μm in diameter and less than 40 μm in diameter, respectively. The following parameters are reported as mean values ± standard deviation (SD) for the adenosine-treated group (n = 13) and the placebo-treated group (n = 14): (a) anagen hair ratio; (b) anagen hair growth rate; (c) thick hairs ratio; (d) thin hair ratio; and (e) hair density. Asterisks with or without a horizontal line indicate P-values from the baseline by a paired two-tailed Student's t-test and between groups by an unpaired two-tailed Student's t-test, respectively. $^{*}P < 0.05$; $^{**}P < 0.01$.

month 12 compared to the baseline (Fig. 1b, P = 0.0143 and P = 0.0310, respectively). Regarding changes of the anagen hair growth rate, the adenosine-treated group was significantly greater than the placebo-treated group (data not shown). The thick hair ratio of the placebo-treated group was significantly reduced over time compared to the baseline (Fig. 1c, P = 0.0064 at month 6 and P = 0.0017 at month 12). This resulted in a significantly higher thick hair ratio in the adenosine-treated group compared with the placebo group (P = 0.0405). Regarding changes of the thick hair rate, the adenosine-treated group was also significantly greater than the placebo-treated group (data not shown).

Regarding the self-assessments, the adenosine-treated subjects were impressed by the efficacy of the lotion on some questionnaire items (Table 2). The adenosine group was significantly greater than the placebo group regarding: (i) the change in appearance of hair at month 12; (ii) the change in hair growth at month 6; and (iii) prevention of hair loss at months 6 and 12. No adverse events were diagnosed in any case based on the safety evaluation.

DISCUSSION

In this study, adenosine showed a significant improvement of FPHL in Japanese women by dermatologist and investigator assessments and by self-assessments. The phototrichogram results also suggested that adenosine had a significant effect on anagen hair growth rate and thick hair rate. FPHL is considered to result from a reduction in hair density and by a miniaturization of hair follicles.[2] In Japanese women, severe hair loss is relatively rare,[13] and the major causes of FPHL might be reductions in hair density and in thick hair ratio.[9] That hypothesis is supported by the present study which shows a reduction in thick hair ratio, and tendencies for reduced anagen hair ratio and hair density in the placebo-treated group (Fig. 1). Our results support the hypothesis that adenosine prevents and/or improves hair loss by maintaining or increasing thick hairs in women with FPHL. We have proposed that the effects of minoxidil are mediated via the expression of VEGF upon activation of adenosine receptors in cultured dermal papilla cells.[7] Adenosine also upregulates the production of fibroblast growth factor 7 (FGF-7)

Table 2. Subject self-assessments†

Question	*P*-value (Mann–Whitney *U*-test)	
	Month 6	Month 12
Bald spot getting smaller since start of study	0.176	0.139
Change in appearance of hair since start of study	0.148	0.048*
Change in hair growth since start of study	0.041*	0.081
Prevention of hair loss since start of study	0.017*	0.036*
Satisfaction with frontal hairline compared to start of study	0.138	0.176
Satisfaction with hair on top of head compared to start of study	0.746	0.146
Satisfaction with hair overall compared to start of study	0.223	0.061

†At 6 and 12 months, subjects self-assessed the conditions of their scalp hair using a questionnaire. Comparisons between the adenosine group (n = 13) and the placebo group (n = 14) were statistically analyzed using Mann–Whitney *U*-tests.*Significant at $P < 0.05$.

by stimulating adenosine receptor A2b in dermal papilla cells.[14] On the other hand, reported inhibitory factor transforming growth factor (TGF)-β[15] was decreased by stimulating the adenosine receptor in dermal papilla (data not shown). We suggest that adenosine promotes hair growth by modulating the expression of genes encoding growth factors such as FGF-7 and VEGF, or TGF-β in dermal papilla cells.

In conclusion, adenosine improves hair loss in Japanese women by stimulating hair growth and by increasing thicker hairs. Therefore, lotions containing adenosine should be useful to treat FPHL in women as well as androgenetic alopecia in men.

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